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Comparative Studies on the Properties of Peroxidase Partially Purified from Different Parts of *Carica papaya*

Roy Emochone Yohanna[1], Mida Habila Mayel[1, *], Richard-Harris Nsenreuti Boyi[1], Sabinus Oscar Onyebuchi Eze[2]

[1]Department of Biochemistry, Federal University Wukari, Wukari, Nigeria

[2]Department of Biochemistry, University of Nigeria, Nsukka, Nigeria

Email address

mayel.mida@fuwukari.edu.ng (M. H. Mayel)

[*]Corresponding author

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Abstract

Peroxidase (E.C.1.11.1.7) is an enzyme with wide industrial and biotechnological applications. However, the high cost of commercially available peroxidase places a barrier to its utilization in so many places in the world. *Carica papaya* Linn. (Pawpaw) has been known as a good source of peroxidase. This study was aimed at identifying the part of papaya with the highest concentration of peroxidase. Peroxidase was extracted from different parts (fruit, leaf and stem) of papaya plant and partially purified through ammonium sulphate precipitation. Peroxidase activity was measured as the change in absorbance due to the oxidation of O-dianisidine in the presence of H_2O_2. The V_{max} and K_m were determined from Lineweaver-Burk plot. Highest peroxidase activity was observed after ammonium sulphate precipitation at 60%, 50% and 70% for the fruit, leaf and stem, respectively. The pH and temperature optima for peroxidase were found to depend on the part of the plant: fruit (pH 6.5, 60°C), leaf (pH 7.0, 40°C) and stem (pH 5.5, 70°C). Peroxidase activity in the leaf (38.4 U/ml) was found to be higher than in the stem (8.6 U/ml) and the unripe *Carica papaya* fruit (0.74 U/ml). From the Lineweaver-Burk plot, peroxidase from the fruit had larger V_{max} and K_m values (V_{max} = 0.678 µmol/min, K_m = 0.63 µmol/min) than both from the leaf (V_{max} = 0.77 µmol/min, K_m = 0.57 µmol/min) and the stem (V_{max} = 0.608 µmol/min, K_m = 0.171). Hence, the leaf of *Carica papaya* can serve as cheap source of peroxidase for industrial application.

Keywords

Enzyme Activity, Biotechnology, Peroxidase, Bioremediation, Lipid Peroxidation, Catalysts

1. Introduction

Peroxidase (E.C.1.11.1.7) is a ubiquitous enzyme which belongs to the oxidoreductase class of enzyme and generally catalyzes a reaction between H_2O_2 as electron acceptor and many kinds of substrates by means of O_2 liberation from H_2O_2 [1]. Peroxidases have been classified into heme and non-heme peroxidases [2, 3]. Peroxidases are versatile, widely distributed in nature and useful biological tools. Over 80% of known peroxidases are heme-containing. Non-heme peroxidases such as thiol-peroxidase, alkylhydroperoxidase, NADH-peroxidase constitute only a small proportion. Two superfamilies of heme peroxidases are peroxidase-cyclooxygenase superfamily (PCOXS) and the peroxidase-catalase superfamily-PCATS [4].

Peroxidases have wide industrial and biotechnological applications [4]. They have been used in bioremediation of harmful pollutants such as humic substances, phenolic compounds, aromatic amines, and dyes [5, 6, 7]. Peroxidases have been used for delignification in paper and pulp industry, hair dyeing, diagnosis kit development, immunoassay, organic and polymer synthesis as well as in enzyme-based biosensor technology and determination of the extent of lipid

peroxidation in meat food products [8, 9, 3]. These diverse possibilities reflect the broad substrate specificity and high specific activity of this natural enzyme.

However, the high cost of commercially available peroxidase places a barrier to its utilization in so many places in the world [10]. In the recent past, research has been geared towards identifying better sources of peroxidases [11]. There are different sources of peroxidase, including plants, microorganisms and animals [12, 13, 14, 15]. Plant peroxidases appear to be very common. There are over 138 members of peroxidases reported in rice, potato tubers, potatoes, horseradish turnip, sour lime, soybean, carrot, wheat, pears, apricot, bananas, fig tree, Arabidopsis, sorghum, and many other plants sources [2, 16, 17, 3]. Peroxidases have been isolated from papaya, tomato, garlic and have been applied in various areas of life for the benefit of man [5, 3, 15].

Carica papaya Linn. (Pawpaw) is a large, single–stemmed herbaceous perennial tree having 20–30 feet height with nutritional, pharmacological and medicinal uses and it has been known as a good source of peroxidase [18]. Peroxidases are the key enzymes of defense related pathways in plants and they play core role in response to wide range of pathogens [16]. Pandey and co-workers mentioned *Carica papaya* as a source of peroxidase and the structure was elucidated afterward [19, 20, 21]. However, due to increased interest in the knowledge of which part of the plant has the highest concentration of peroxidase, this study was carried out to identify the part of *C. papaya* with the highest concentration of peroxidase.

2. Materials and Methods

2.1. Plant Materials

Plant materials (leaves, stem and unripe fruits of *Carica papayai*) were obtained from Margaret Cartwright Avenue, University of Nigeria, Nsukka by cutting down a young *C. papaya* plant and removing the leaves, stem and unripe fruits.

2.2. Chemicals and Reagents

BSA-Bovine Serum Albumin, O-dianisidine, Folin-Ciocalteau Phenol Reagent were purchased from Sigma-Aldrich, USA. All other chemicals and reagents used in this study (Absolute Ethanol, Na_2CO_3 and NaOH, $CuSO_4.5H_2O$, Sodium-Potassium Tatarate, HCl, Acetic Acid, H_2O_2, sodium acetate, etc) were of analytical grade.

2.3. Preparation of Crude Peroxidase Extracts

Extraction of the enzyme was done by the modified method of Eze [12]. 100g of the sample (fruit, leaf, and stem respectively) was washed separately in clean water and chopped into small pieces with a knife and ground in a hand grinder and subsequently refrigerated. The environment of the grinder was fitted with ice block to maintain a cold temperature. 500g of the cold Tris-Hcl buffer was used to wash the sample and filtered through four-layered cheese cloth. It was then centrifuged at 10,000g for 30 min at 4°C. The supernatant was collected as the crude enzyme and stored frozen until used. Peroxidase was partially purified by ammonium sulphate precipitation.

2.4. Peroxidase Assay (Using O-Dianisidine) and Protein Determination

Peroxidase assay was done by the method described by Eze [12]. The change in absorbance at 460nm due to the inactivation of O-dianisidine in the presence of hydrogen peroxide and the enzyme extract at 30°C was monitored using Jenway UV-Vis Spectrophotometer (Model 721) after every 30 sec for 3min. The standard assay solution contained 2.7 ml sodium acetate buffer, pH 6.5, 0.1 ml of 0.2% O-dianisidine and 0.1 ml of 30% H_2O_2 and 0.1ml of suitably diluted enzyme extract in a total of 3ml. The absorbance was read at 460 nm using Spectrophotometer (Model 721) after 30 sec for 3min.

One unit of enzyme activity was defined as the amount of enzyme that gives an absorbance change of 0.1AU/min at 30°C. Protein concentration was determined by the method described by Lowry [22], using bovine serum albumin-BSA as standard.

2.5. Characterization of Enzyme

Dialyzed peroxidase samples were characterized with respect to effect of pH, temperature, substrate concentration on the activity of the enzyme. The experiments were done in replicates and the mean values of the absorbance used to calculate the activity.

The effect of pH on the activity of the enzyme was studied by measuring the enzyme activity at various pH values in the range of 3-9.5 at 0.5 intervals. The optimum temperature of peroxidase was investigated by measuring enzyme activity within a temperature range of 10-100°C at intervals of 10°C. Incubation of the reaction mixture was in a thermostatic water-bath, Model SSY-H.

The effect of substrate concentration on the activity of peroxidase was studied by determining peroxidase activity at different concentrations of H_2O_2 (0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, 0.6 ml, 0.7 ml, 0.8 ml, 0.9 ml and 1.0 ml) and o-dianisidine (10 mg/ml, 30 mg/ml, 50 mg/ml 70 mg/ml, and 90 mg/ml, 100 mg/ml) at the pH and temperature optima. Kinetic parameters (V_{max} and K_m) were then determined from the Lineweaver-Burk plots and comparisons made.

3. Results

3.1. Purification of Peroxidase

Peroxidase from different parts of *Carica papaya* was partially purified through ammonium sulphate precipitation and dialysis. Ammonium sulphate concentrations for fruit, stem, and leaf peroxidase precipitation were 60%, 70%, and 50% respectively. Peroxidases of papaya fruit, stem, and leaf were respectively purified to 0.112, 0.686, and 2.560 folds.

3.2. Effect of pH on Peroxidase Activity

The pH optima for peroxidase from the different parts of papaya were 6.5 for the fruit, 7.0 for the leaf, and 5.5 for the stem. The result is shown in figure 1 below

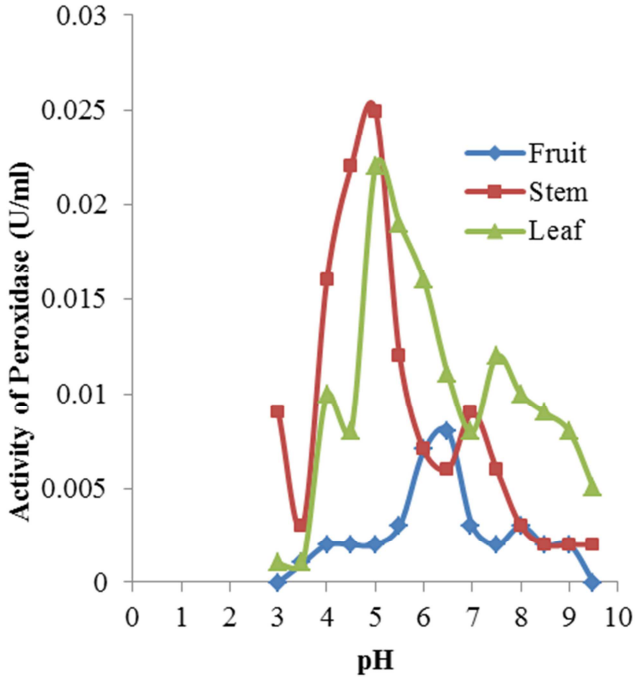

Figure 1. *Effect of pH on peroxidase activity of Carica papaya.*

3.3. Effect of Temperature

The temperature optima for peroxidase were determined at their respective optimal pH values. The temperature optima for peroxidase from fruit, stem and leaf were 60°C, 70°C and 40°C respectively. See figure 2 below.

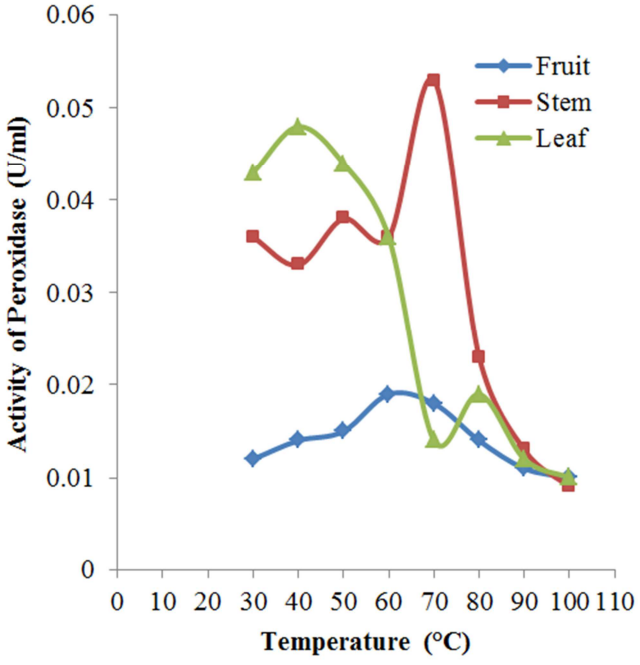

Figure 2. *Effect of temperature on peroxidase activty of Carica papaya.*

3.4. Determination of Kinetic Parameters

Kinetic parameters, V_{max} and K_m, were calculated from the double reciprocal plots for the peroxidase of the fruit, leaf and stem. For peroxidase of fruit, the V_{max} was 0.678 U/ml and K_m = 0.63 µmol/ml whereas for leaf peroxidase, V_{max} is 0.77 U/ml and K_m = 0.57 µmol/ml. Stem peroxidase had V_{max} of 0.608 U/ml and K_m of 0.171 µmol/ml. The results are shown in the figures 3, 4 and 5 below.

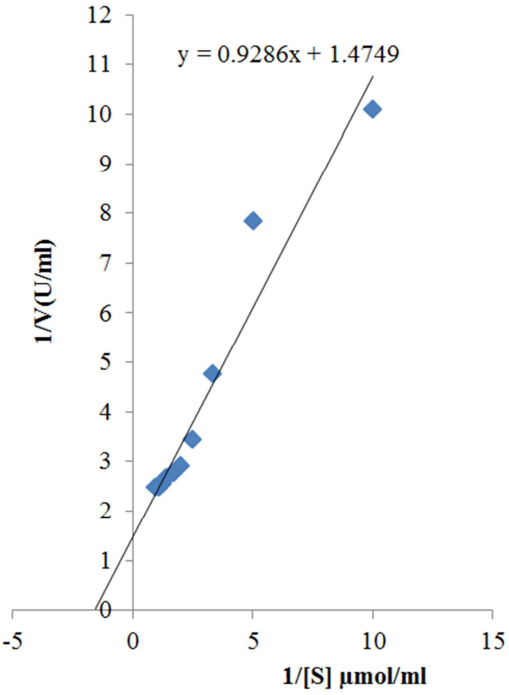

Figure 3. *Line-Weaver Bulk plot of effect of substrate concentration on peroxidase from the fruit of Carica papaya.*

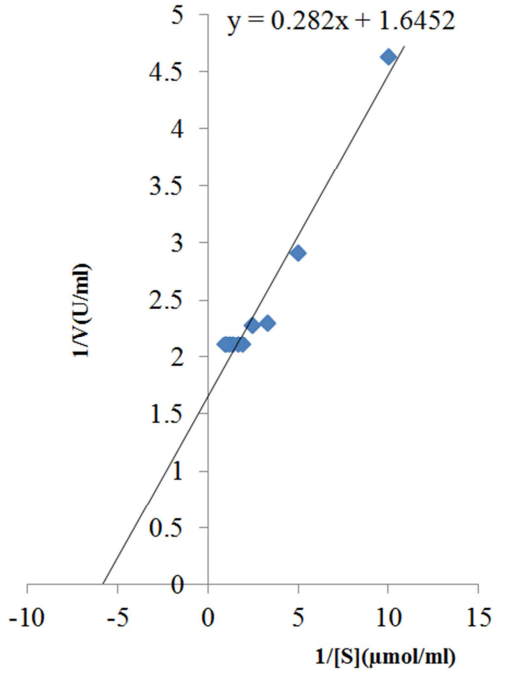

Figure 4. *Line-Weaver Bulk plot of effect of substrate concentration on peroxidase from the stem of Carica papaya.*

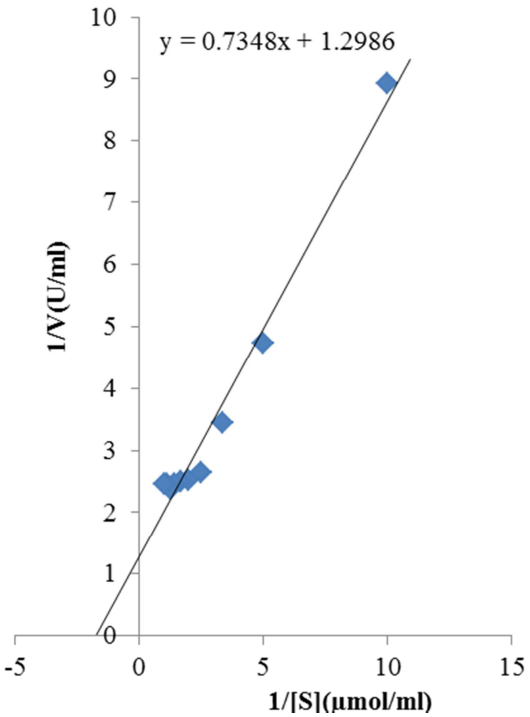

Figure 5. *Line-Weaver Bulk plot of effect of substrate concentration on peroxidase from the leaf of Carica papaya.*

4. Discussion

Peroxidases from different parts of *Carica papaya* were precipitated by different concentrations of ammonium sulphate. Precipitation of peroxidase from stem at 70% shows that it is more hydrophilic than those of leaf and fruit (precipitated at 50 and 60% respectively). These results are in agreement with those in the literature [7, 23]. The precipitation was likely as a result of dehydration of the microenvironments of the protein molecules (by the salts), leading to more protein-protein interaction or self aggregation. This is because the salt molecules (with higher charge) are more hydrophilic than the proteins and so withdraw the water molecules from protein-water interactions. This ultimately results in protein precipitation or salting out, however, the salt concentration at which a protein precipitates varies with the number of hydrophilic aminoacyl residues in a protein [24].

Assay for peroxidase activity in fruit, leaf and stem showed that peroxidase activity depends on the pH of the environment [25, 26]. It is known that the optimum pH of an enzyme varies according to source and substrate types [27]. From this study, the pH optima for peroxidase activity from fruit, leaf and stem were 6.5, 7.0, and 5.0, respectively. Similar results have been reported by other researchers [7, 25, 23, 26]. At pH optima, enzyme active sites have maximum interaction with their substrates giving best activities. Drifts in pH below or beyond the optima disrupt the functional (three-dimensional) structures of the enzymes, resulting in the loss of activity [28-30]. Changes in pH may not only affect the shape of an enzyme but also change the shape or charge properties of the substrates so that they either fail to bind to the active site or undergo catalysis. In general, enzymes have pH optima; however the optimum is not the same for each enzyme [31-33].

At pH optima, peroxidase activity increased concurrently with increase in temperature. Temperature optima for the enzymes in the samples were 60°C (fruit), 40°C (leaf), and 70°C (stem). Previous studies on peroxidase have typically determined an optimum temperature for peroxidase in the range of 30 to 70°C [34, 35, 26, 36]. Temperature disrupts the structure of enzymes by increasing the kinetic energy of vibrating atoms and functional groups, thereby breaking weak molecular bonds [32, 33]. Our results suggest that peroxidase from the stem of papaya will be more suitable for industrial processes that require higher temperatures.

From the kinetic parameters determined in our study, we found leaf peroxidase to have a higher V_{max} (0.77 U/ml) than those of fruit peroxidase (V_{max} = 0.678 U/ml) and stem peroxidase (V_{max} = 0.608 U/ml). Values within this range have been reported in the literature [37, 38, 26]. Our results indicate that peroxidase from papaya leaf has higher rate of conversion of substrates to products and so will be more suitable for industrial purposes, with respect to speed of reactions. Pertaining K_m, papaya stem peroxidase reported a lower (K_m = 0.171 µmol/ml) value than both the leaf (K_m = 0.57 µmol/ml) and fruit (0.63 µmol/ml) peroxidases. Similar results have been reported by other researchers [38, 26]. The lower K_m of stem peroxidase indicates that a small quantity of the substrate could saturate the enzyme and hence the enzyme is more specific for the substrate than peroxidase of the leaf and fruit.

5. Conclusion

Carica papaya leaves, having the highest concentration of peroxidase than the fruits and stem of the plant, can be explored as a cheap source of peroxidase for biotechnological purposes. We recommend that this peroxidase be purified to higher level and the molecular weight determined. Structural elucidation of this enzyme will also reveal more knowledge about its mechanism of action.

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