

Musa paradisiaca stem juice as a source of peroxidase and ligninperoxidase

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Musa paradisiaca stem juice has been shown to contain peroxidase activity of the order of 0.1 enzyme unit/ml. The K_m values of this peroxidase for the substrates guaiacol and hydrogen peroxide are 2.4 and 0.28 mM respectively. The pH and temperature optima are 4.5 and 62.5°C respectively. Like other peroxidases, it follows double displacement type mechanism. At low pH, *Musa paradisiaca* stem juice exhibits ligninperoxidase type activity. The pH optimum for ligninperoxidase type activity is 2.0 and the temperature optimum is 24°C. The K_m values for veratryl alcohol and n-propanol are 66 and 78 μ M respectively.

Peroxidases [EC 1.11.1.7] are heme containing enzymes found in plants, in some animal tissues and in microorganisms¹. They perform a variety of physiological functions like lignification of cell wall and in defense mechanism against pathogenic attacks². Some of the peroxidases play crucial roles in delignification of lignocellulosic materials³ and in degradation of recalcitrant organic pollutants⁴. Recent studies have revealed that not all peroxidases are similar in their structures and functions^{2, 5-11}. Ligninperoxidase differs from horseradish peroxidase in the sense that ligninperoxidase directly oxidises veratryl alcohol whereas horseradish peroxidase can not⁷. Soyabean peroxidase⁷ has ligninperoxidase type activity but it is more stable at acidic pH and at higher temperatures than the ligninperoxidase. These studies have indicated that peroxidases from different sources should be studied to find their biocatalytic potential¹². Keeping this point in view, we have analysed the *Musa paradisiaca* stem juice for peroxidase activity and have found that it is a good source of peroxidase. Moreover, *M. paradisiaca* stem juice contains ligninperoxidase activity as well.

Materials and Methods

Veratryl alcohol was from Aldrich (USA) and guaiacol was from Sigma (USA). All other chemicals were from S.d. fine chemicals (Mumbai) and were used without further purification.

The enzyme was isolated by cutting the stem of *Musa paradisiaca* into small pieces, crushing the

pieces in mortar with pestle and extracting the juice by keeping the pieces in four layers of cheese cloth and squeezing it. The juice was centrifuged using Sigma (Germany) model 3K 30 refrigerated centrifuge at 4000 g for 20 min at 4°C to remove the cloudiness of the juice. The clear juice was concentrated 20 times in an Amicon (USA) Concentration Cell model 8200 using PM 10 ultrafiltration membrane with molecular weight cut off value of 10,000. The concentrated enzyme was stored at 4°C. The enzyme stored in this way does not lose any activity even after 4 months.

Peroxidase activity of the enzyme was measured in 50 mM sodium phosphate buffer pH 7.0 at 30°C using guaiacol 5 mM, hydrogen peroxide 0.6 mM as the substrates and by monitoring the absorbance changes at 470 nm using molar extinction coefficient value of $2.66 \times 10^4 M^{-1} cm^{-1}$ for the product tetraguaiacol formed by the enzymatic reaction¹³.

Ligninperoxidase activity of the enzyme was tested using veratryl alcohol 2 mM, hydrogen peroxide 0.4 mM at 30°C by monitoring absorbance changes at 310 nm due to the formation of the product veratraldehyde and using molar extinction coefficient value of $9300 M^{-1} cm^{-1}$ ¹⁶. Lignolytic activity has also been tested using a newly reported method¹⁵ in which n-propanol has been used in place of veratryl alcohol and the rate of the formation of the product propanal has been monitored at 300 nm using molar extinction coefficient value of $20 M^{-1} cm^{-1}$. Hydrogen peroxide used in both the above cases was freshly prepared each time by measuring absorbance at 240 nm using molar extinction coefficient value of $39.4 M^{-1} cm^{-1}$

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and suitably diluting the solution. All spectrophotometric measurements were done with UV/VIS spectrophotometer Hitachi (Japan) model U-2000 which was fitted with electronic control unit for variation of temperature in the cuvettes. The least count of the absorbance measurement was 0.001 and one enzyme unit is the amount of enzyme which produces 1 $\mu\text{mol}/\text{min}$ of the product.

Results and Discussion

Figure 1(a) is a typical plot showing the variation of absorbance at $\lambda=470$ nm vs time in a peroxidase assay solution containing *M. paradisiaca* stem juice. Fig. 1(b & c) are corresponding plots for the same assay solutions containing no juice and juice which was denatured by boiling in water for 1 hr respectively. In case of assay solution containing active juice absorbance at $\lambda=470$ nm increases with time whereas in cases of assay solutions containing no juice or juice which was denatured by boiling, absorbance does not change with time indicating clearly that *M. paradisiaca* stem juice contains peroxidase activity. The calculation has shown that the juice contains approximately 0.1 enzyme unit/ml of the juice indicating that it is a good source of peroxidase enzyme for biotransformations¹².

Musa paradisiaca stem juice peroxidase has been tested for Michaelis-Menten type kinetics using guaiacol as the variable substrate at the saturating con-

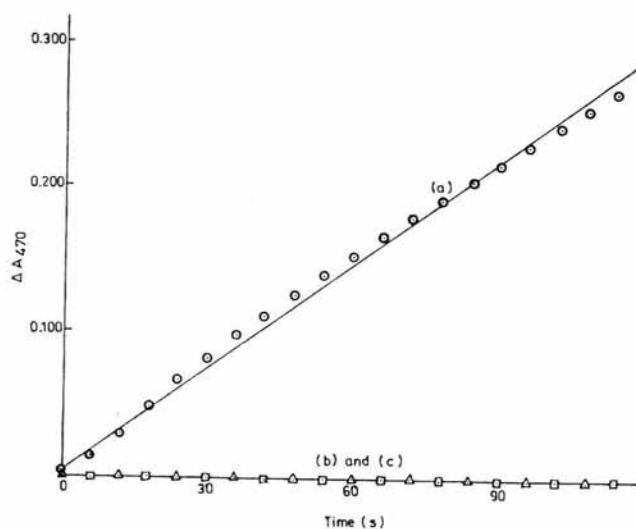


Fig.1—Peroxidase activity of *M. paradisiaca* stem juice. Assay solution contains 5 mM guaiacol, 1 mM hydrogen peroxide in 50 mM sodium phosphate buffer pH 4.5 at 30°C and 20 μL of enzyme stock having 0.1 enzyme unit/ml has been added. (a) Active enzyme (O), (b) boiled enzyme (Δ) and (c) no enzyme ().

centration of hydrogen peroxide and also using hydrogen peroxide as the variable substrate at the saturating concentration of guaiacol. Double reciprocal plots in both the cases are straight lines showing that the reported peroxidase obeys Michaelis-Menten kinetics¹⁷. The calculated K_m values for guaiacol and hydrogen peroxide are 2.4 mM and 0.28 mM respectively. The corresponding values of K_m for horseradish peroxidase¹⁶ are 0.8 and 0.1 mM respectively indicating that the reported enzyme has lower affinity for both the substrates in comparison to horseradish peroxidase, a plant peroxidase which has been studied extensively. Since peroxidases are known to follow double displacement type kinetics¹⁷, *Musa paradisiaca* stem juice peroxidase was also analysed for this type of kinetics by measuring steady state velocity of the enzyme catalyzed reaction at three different fixed concentrations of hydrogen peroxide and varying the concentration of guaiacol at each hydrogen peroxide concentration and also at three different fixed concentrations of guaiacol and varying the concentration of hydrogen peroxide. Double reciprocal plots in both the cases have been found to be parallel straight lines confirming that the reported peroxidase also follows double displacement type mechanism observed in case of other peroxidases¹⁷.

In order to find the optimum conditions for the functioning of this peroxidase as an efficient biocatalyst, the activities of this peroxidase at varying pH and varying temperature have been studied. The peroxidase has pH optimum around 4.5 and temperature optimum around 62.5°C (Figs 2 & 3).

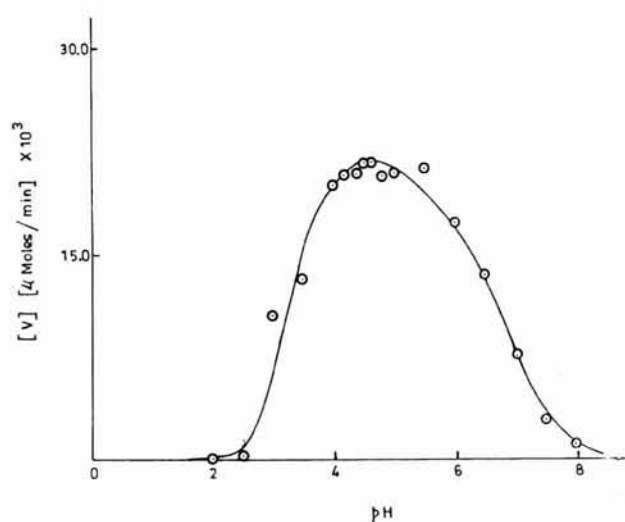


Fig.2—SEM micrograph of C/Poly(1,5-DHN) electrode prepared under potentiodynamic condition.

Recently,^{2,7} some plant peroxidases have been shown to exhibit liginperoxidase type activity and can oxidise veratryl alcohol directly in presence of hydrogen peroxide at low pH. The *M. paradisiaca* stem juice was also analysed for liginperoxidase type

activity at pH 2.0 using veratryl alcohol as the substrate. A typical plot showing the increase of absorbance at 310 nm with time due to formation of veratraldehyde is shown in Fig. 4(a). Recently it has been reported¹⁵ that n-propanol can also be used for assay-

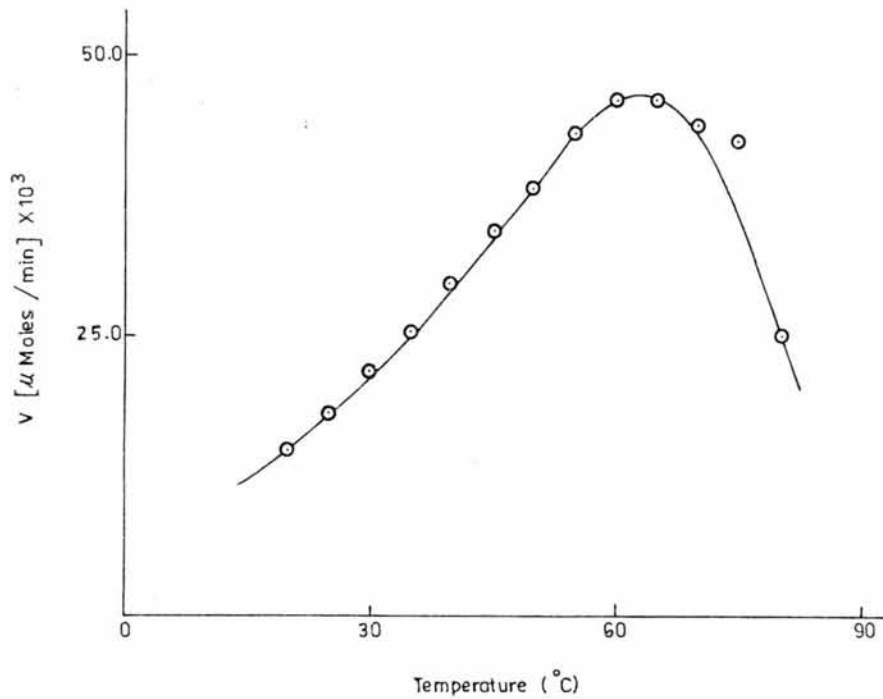


Fig.3—Dependence of the enzyme activity on temperature of the assay solution. Assay solution composition is the same as mentioned in the legend to Fig.1 except the temperature of the reaction medium which has been varied.

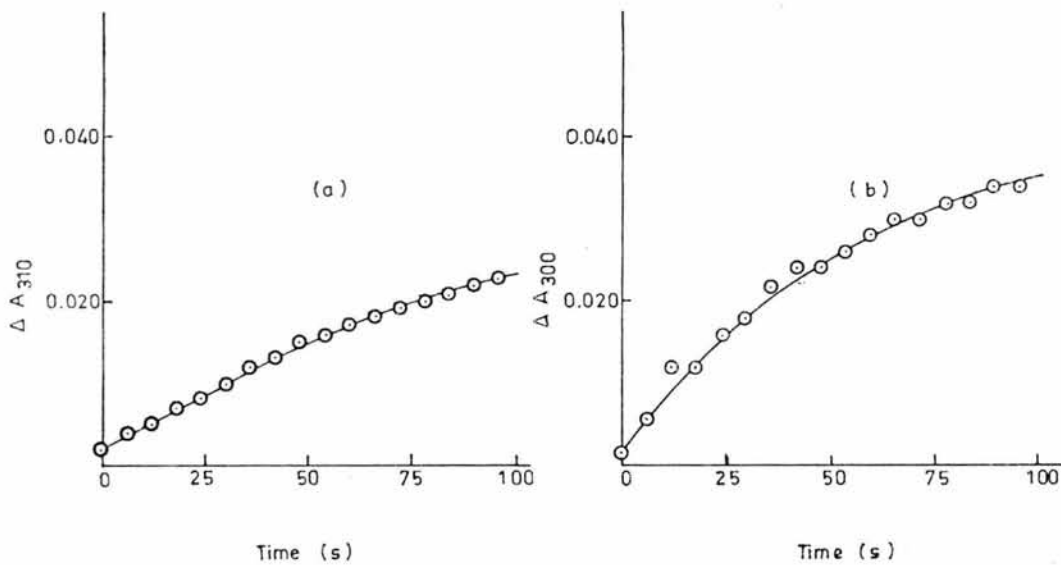


Fig.4—Liginperoxidase activity of *M. paradisiaca* stem juice using veratryl alcohol and n-propanol as different substrates. (a) Assay solution contains 2 mM veratryl alcohol, 0.4 mM hydrogen peroxide in 50 mM sodium phosphate buffer pH 2.0 at 30°C and 20 μL of concentrated juice has been added. (b) Assay solution composition is the same as in case (a) except the veratryl alcohol which has been replaced by 3.25 mM n-propanol.

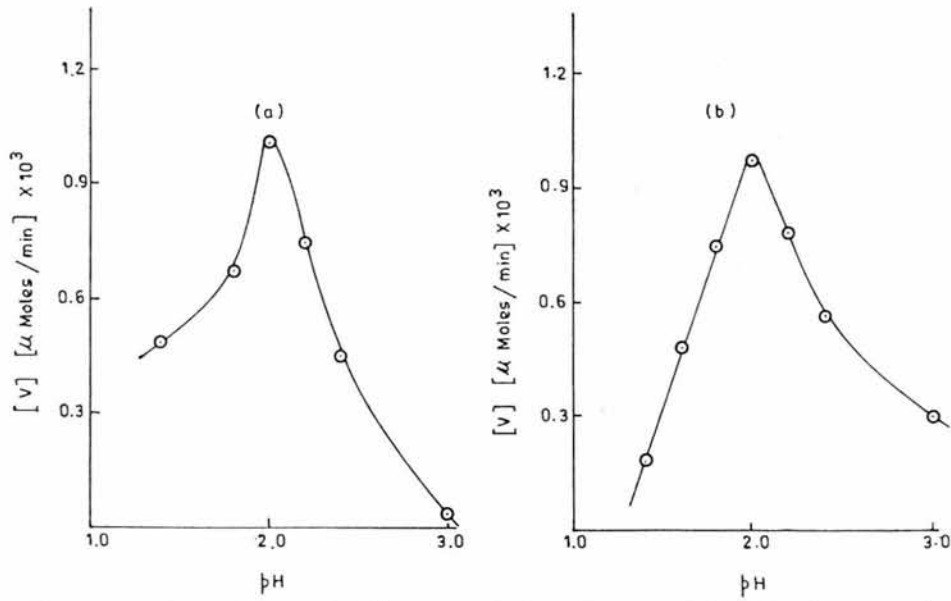


Fig.5—Dependence of ligninperoxidase activity on pH of the assay solution using veratryl alcohol and n-propanol as different substrates. Assay solution composition is the same as mentioned in the legend to Figs. 4 (a) and 4 (b) respectively except the pH of the reaction medium which has been varied. (a) Veratryl alcohol, (b) n-propanol.

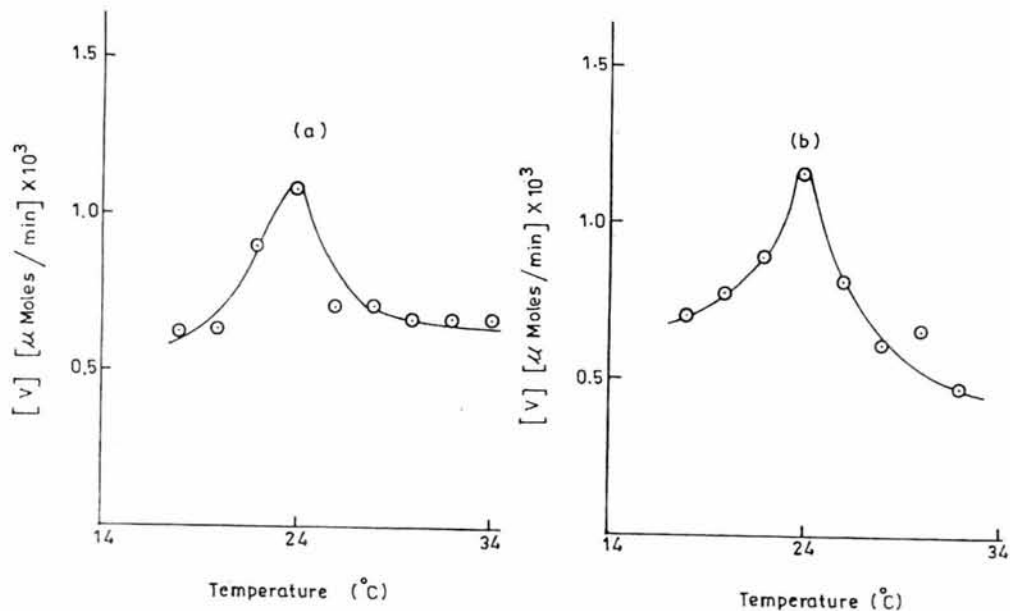


Fig.6—Dependence of ligninperoxidase activity on temperature of the assay solution using veratryl alcohol and n-propanol as different substrates. Assay solution composition is the same as mentioned in the legend to Figs. 4 (a) and 4 (b) respectively except the temperature of the reaction medium which has been varied. (a) Veratryl alcohol, (b) n-propanol.

ing the ligninperoxidase type activity in place of veratryl alcohol and the absorbance change at 300 nm can be monitored. This newly reported¹⁵ method was also used for assaying the ligninperoxidase type activity of *M. paradisiaca* stem juice. The typical plot is shown in Fig. 4(b). It is obvious from both the Fig. 4(a and b) that the stem juice has ligninperoxidase type activity as well.

Michaelis-Menten type kinetics for ligninperoxidase type activity of *M. paradisiaca* stem juice has been tested using veratryl alcohol and n-propanol as the variable substrates in different experiments at the saturating concentrations of hydrogen peroxide. The double reciprocal plots in both the cases are straight lines confirming that the ligninperoxidase type activity follows Michaelis-Menten kinetics¹⁷. The K_m values

for veratryl alcohol and n-propanol are 66 and 78 μM respectively. The pH and temperature optima for this activity have been determined using veratryl alcohol as well as n-propanol as the substrates and monitoring the formation of veratraldehyde and n-propanol at 310 and 300 nm respectively. The results are plotted in Fig. 5 and 6. It is obvious from these figures that pH optimum for linginperoxidase type activity is 2.0 and temperature optimum is 24°C. The most extensively studied linginperoxidase activity is from *Phanerochaete chrysosporium* which has pH optimum around 3.0 and temperature optimum around 26°C. The reported pH optimum for the linginperoxidase type activity of soyabean peroxidase⁷ is 2.4 and of tobacco peroxidase² is 1.8. Thus pH optimum of linginperoxidase type activity of *M. paradisiaca* stem juice is in the same range as reported for linginperoxidase type activity from soyabean⁷ and tobacco² sources. The temperature optimum of linginperoxidase type activity from soyabean and tobacco sources is not reported and hence a comparison can not be made.

Recently Adam *et al.*¹² have reviewed the literature on the synthetic applications of peroxidases which include hydroxylation of arenes, the oxyfunctionalization of phenols and aromatic amines, the epoxidation and halogenation of olefins, the oxygenation of hetero atoms and the enantioselective reduction of racemic hydroperoxides. We report here a convenient and rich source of peroxidase which can be tested for the above conversions.

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References

- Rodriguez - Lopez J N, Smith A T & Thorneley R N F, Recombinant horseradish peroxidase isoenzyme C: the effect of distal haem cavity mutations (His 42 \rightarrow Leu and Arg 38 \rightarrow Leu) on compound I formation and substrate binding, *J Biol Inorg Chem*, 1 (1996) 136.
- Gazarian I G, Lagrimini L M, George S J & Thorneley R N F, Anionic tobacco peroxidase is active at extremely low pH: veratryl alcohol oxidation with a pH optimum of 1.8, *Biochem J*, 320 (1996) 369.
- Tien M & Kirk T K, Lignin - degrading enzyme from *Phanerochaete chrysosporium*: purification, characterization and catalytic properties of a unique H₂O₂-requiring oxygenase, *Proc Natl Acad Sci USA*, 81(1984)2280.
- Bumpus J A, Tien M, Wright D & Aust S D, Oxidation of persistent environmental pollutants by a white rot fungus, *Science*, 228 (1985) 1434.
- Kvaratskhelia M, Winkel C & Thorneley R N F, Purification and characterization of a novel class III peroxidase isoenzyme from tea leaves, *Plant Physiol*, 114 (1997) 1237.
- Gazaryan I G & Lagrimini L M, Purification and unusual kinetic properties of a tobacco anionic peroxidase, *Phytochemistry*, 41 (1996) 1029.
- Mc Eldoon J P, Pokora A R & Dordick J S, Linginperoxidase type activity of soybean peroxidase, *Enzyme Microb Technol*, 17 (1995) 359.
- Finzel B C, Poulos T L & Kraut J, Crystal structure of yeast cytochrome C peroxidase refined at 1.7Å³ resolution, *J Biol Chem*, 259 (1984)13027.
- Poulos T L, Edwards S L, Wariishi H & Gold M H, Crystallographic refinement of linginperoxidase at 2 Å³, *J Biol Chem*, 268 (1993) 4429.
- Sundaramoorthy M, Kishi K, Gold M H & Poulos T L, The crystal structure of manganese peroxidase from *Phanerochaete chrysosporium* at 2.06 Å³ resolution, *J Biol Chem*, 269 (1994) 32759.
- Patterson W R & Poulos T L, Crystal structure of recombinant pea cytosolic ascorbate peroxidase, *Biochemistry*, 34 (1995) 4331.
- Adam W, Lazarus M, Saha-Moller C R, Weichold O, Hoch U, Haring D & Schreier P, Biotransformations with peroxidases, *Adv Biochem Eng / Biotechnol*, 63 (1999) 73.
- Whitaker J R, Principles of enzymology for food sciences (Marcel Dekker Inc New York) 1972.
- Tien M & Kirk T K, *Meth Enzymol*, 161 (1988) 238.
- Shanmugam V, Kumari M & Yadav K D S, n-propanol as a substrate for assaying the linginperoxidase activity of *Phanerochaete chrysosporium*, *Indian J Biochem Biophys*, 36 (1999) 39.
- Rodriguez - Lopez J N, Smith A T & Thorneley R N F, Role of arginine 38 in horseradish peroxidase: a critical residue for substrate binding and catalysis, *J Biol Chem*, 271 (1996) 4023.
- Fersht A, Enzyme structure and mechanism (2nd ed) (W H Freeman and Co Reading and San Francisco) 1985.