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Purification of lignin peroxidase from the juice of *Musa paradisiaca* stem

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ABSTRACT

The purification of the lignin peroxidase from the juice of *Musa paradisiaca* stem using a simple procedure involving concentration by ultrafitration and anion exchange chromatography on DEAE cellulose column has been reported.The enzymatic properties of the purified enzyme have been found to be similar to the enzymes reported from the fungal sources.

Key words: Peroxidase, metalloenzymes, lignin, *Musa paradisiaca.*

INTRODUCTION

Peroxidases [EC 1.11.1.7] are heme containing enzymes found in plants, in some animal tissues and in microorganisms[1].They perform a variety of physiological functions like lignification of cell wall and in defense mechanism against pathogenic attacks[2].Some of the peroxidases play crucial roles in delignification of lignocellulosic materials[3] and in degradation of recalcitrants organic pollutants[4]. Recent studies have revealed that not all peroxidases are similar in their structures and functions[2, 5-11].Lignin peroxidase differs from horseradish peroxidase in the sense that lignin peroxidase directly oxidizes veratryl alcohol whereas horseradish peroxidase can not[7].Soyadean peroxidase[7] has lignin peroxidase type activity but it is more stable at acidic pH and at higher temperatures than the lignin peroxidase.These studies have indicated that peroxidases from different sources should be studied to find their biocatalytic potential[12].Vernwal et al.[13] have reported the lignin peroxidase activity in Musa paradisiacal stem juice but the enzyme has not been purified to homogeneity. In this communication, purification of lignin peroxidase from the juice of *M. parasidiaca* stem has been reported.

MATERIAL AND METHODS

Chemicals

DEAE cellulose was from Sigma Chemical Company, St. Louis (USA) and veratryl alcohol, 3,4-dimethoxy benzyl alcohol was from Aldrich Chemical Company, Inc. Wiscosin (USA).All the chemicals including molecular weight markers, phosphorylase (97.4 kDa), bovine serum albumin (66 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), soyabean trypsin inhibitor (20.1 kDa) and lysozyme (14.3 kDa) used in SDS-PAGE analysis were procured from Bangalore Genei Pvt. Ltd, Bangalore (India). All other chemicals were either from Merck Ltd., Mumbai (India) or from s.d.fine chem. Ltd., Mumbai (India) and were used without further purifications.

Enzyme assay

The lignin peroxidase activity was assayed by monitoring the formation of veratraldehyde spectrophotometrically at λ= 310nm using veratryl alcohol as the substrate[14] with UV/VIS spectrophotometer Hitachi (Japan) model U-2000 which was fitted with electronic temperature control unit. The molar extinction coefficient value[14] of 9300 M^{-1} Cm^{-1} for veratraldehyde was used for calculating

the enzyme unit which was defined as the amount of enzyme which converts one µmole of the substrate to the product under the standard assay condition. The least count of absorbance measurement was 0.001 absorbance unit. The reaction solution 1 ml consisted of 2 mM of veratryl alcohol, 0.4 mM of H_2O_2 in 50 mM sodium tartrate buffer pH 2.5 at 25°C and a suitable aliquot of the enzyme.

Purification of the enzyme

The enzyme was isolated by washing the stem of M.paradisiaca with milli Q water, cutting it into small pieces, crushing the pieces in mortar with four layers of cheese cloth and squeezing it. The juice was centrifuged using sigma (Germany) refrigerated centrifuge model 3K30 at 4000g for 20 minutes at 4°C to remove the cloudiness of the juice. The clear juice 180 ml was concentrated 30 times using using Amicon concentration cell model 8200 and ultrafiltration membrane PM10 with molecular wt.cut off value of 10 kDa. The concentrated crude enzyme solution 6 ml was dialysed against 6L of 10 mM sodium acetate buffer pH 6.0 for 24 hrs over three changes of the buffer. The dialyzed crude enzyme solution was loaded on a DEAE cellulose column size 1 cm X 33 cm equilibrated with 10 mM sodium acetate buffer pH 6.0 at the flow rate of 16 ml/hr. The bound protein was washed with 100 ml of the same buffer and the protein was eluted with linear gradient of sodium chloride 0-1M in the same buffer (100 ml + 100 ml with 1M NaCl). The 4.0 ml fractions were collected and analysed for the activity of lignin peroxidase[14]. All the fractions were analysed for protein concentration using Lowry method[15].The lignin peroxidase fractions were pooled, concentrated using Amicon concentration cell model 8200 and then model 3 using ultrafiltration membranes PM10.The concentrated enzyme was stored in the fridge at 4°C.

SDS-Polyacrylamide gel electrophoresis

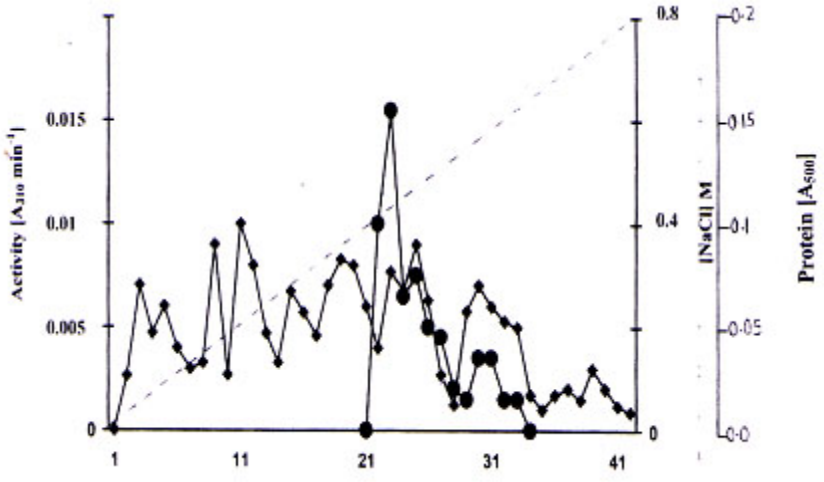
The homogeneity of the purified lignin peroxidase was analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis using the method of Weber and Osborn[16]. The separating gel was 12% acrylamide in 0.375M Tris-HCl buffer pH 8.8 and stacking gel was 5% acrylamide in 0.063M Tris-HCl buffer 6.8. Gel was run at a constant current of 20 mA . Proteins were visualized by staining with coomassie Blue R-250.

Determination of enzymatic characteristics

The $K_{m,}$ pH and temperature optima were determined using veratryl alcohol as the substrate and monitoring the formation of veratraldehyde spectrophotometrically as mentioned in the enzyme assay[14]. For the determination of Km value for veratryl alcohol, steady state velocities of the enzyme catalysed reaction at different concentrations of veratryl alcohol [0.05 to 2.0 mM] and keeping the concentration of H_2O_2 at a fixed enzyme saturating value of 0.4 mM were determined.The Km value was calculated from the linear regression of double reciprocal plot[17]. A similar procedure was adapted for the determination of Km value for H_2O_2. The pH optimum was determined by measuring the steady state velocity of the enzyme catalysed reaction using veratyl alcohol as the substrate in solutions of different pH values [1.4-3.0] which were maintained using 50mM sodium tartrate buffer and plotting the steady state velocity vs pH of the reaction solution .For determination of temperature optimum steady state velocity of the enzyme catalysed reaction was determined at different temperatures [18-34°C] and a plot of steady state velocity vs temperature was made from which the value of temperature optimum was calculated.

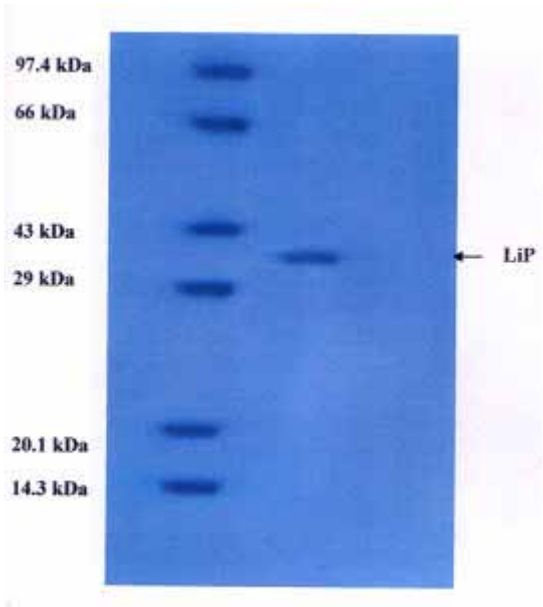
RESULTS AND DISCUSSION

The elution profile of the lignin peroxidase from DEAE cellulose column is shown in Fig.1. All the fractions were analysed for lignin peroxidase activity but the lignin peroxidase activity was found in fractions numbered 21-30 only.All these lignin peroxidase active fractions were combined and concentrated.The results of SDS-PAGE analysis of the concentrated combined lignin peroxidase fractions are shown in Fig. 2. The presence of a single protein band in lane 2 in which purified lignin peroxidase has been loaded clearly shows that the purified lignin peroxidase is homogeneous. The calculated molecular wt of the purified lignin peroxidase is 39 kDa which is in the range reported for the molecular wts of lignin peroxidases of fungal strains[14,18,19]. The Km values for veratryl alcohol and H_2O_2 of the purified enzyme were found to be 66



(•) shows the ativity – profile, (■) shows protein at 500nm, (- - -) Concentration of NaCl gradient

Fig. 1. Typical elution profile from DEAE column



Lane 1 contains the molecular weight markers (from top): Phosphorylase (97.4 kDa), Bovine serum albumin (66 kDa), Ovalbumin (43 kDa),Carbonic anhydrase (29 kDa), Soyabean trypsin inhibitor (20.1 kDa & Lysozyme (14.3 kDa). Lane 2 contains the purified lignin peroxidase.

Fig. 2: **SDS-PAGE analysis**

µM and 78 µM respectively which are near to the values 60 µM and 80 µM reported in case of the lignin peroxidase purified from *Phanerochaete chrysosporium*. The pH and temperature optima of the purified enzyme were 2.0 and 24°C respectively which also are near to the values of 3 and 26°C respectively reported[18] for the lignin peroxidase of *P. chrysosporium*, the ligninperoxidase which has been studied extensively[20,21].

Thus this communication reports the purification of a biotechnologically important enzyme from a conveniently available plant source, *M. paradisiaca* stem. The properties of the enzyme are similar to the properties reported for the lignin peroxidases of fungal sources[14,18,19].

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