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RESEARCH ARTICLE

PARTIAL PURIFICATION OF PEROXIDASE FROM IRAQI RADISH ROOTS.

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Abstract

Peroxidases (EC 1.11.1.7) are enzymes whose primary function is to oxidize a variety of hydrogen donor at the expense of hydrogen peroxide. In the present study, peroxidase was partially purified from Iraqi radish. Crude extract was prepared by blending and centrifugation of local radish roots. The enzyme was salt precipitated using 80% ammonium sulfate, dialyzed and then partially purified using DEAE-Cellulose ion exchange chromatography. Two fractions of peroxidase activity were eluted; the first was purified 35.62 folds and showed a final specific activity 41.85U/mg with a 51.29% yield. The second was purified 23.45 folds and showed a final specific activity 27.551U/mg with a 42.12% yield.

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Introduction:-

Peroxidase (POD) an oxidoreductase is a heme protein, catalyses the oxidation of a wide variety of organic and inorganic substrates using hydrogen peroxide as the electron acceptor (1). Peroxidases are widely distributed in living organisms including microorganisms, plants and animals (2). POD is mainly located in the cell wall (3) and it is one of the key enzymes controlling plant growth and development (4).

From the economical point of view, peroxidase is an important enzyme. POD is widely used for clinical diagnosis and microanalytical immunoassays because of its high sensitivity. It is used in diagnostic kits for enzymatic determination of glucose, uric acid, cholesterol and many other metabolites in biological fluids and is also an important enzyme in ELISA systems (5). Other applications include synthesis of various aromatic compounds and removal of peroxide from foodstuff and industrial wastes (6). It was reported that peroxidase had been used for biotransformation of organic molecules (7). Because of its broader catalytic activity, a wide range of chemicals can be modified using POD. Also, it can be used for the applications such as synthesis of various aromatic compounds, removal of phenolics from waste waters and the removal of peroxides from foodstuffs, beverages and industrial wastes (8).

Although, peroxidases are widely distributed in the plant kingdom, the major source of commercially available peroxidase is roots of horseradish. Many investigators extracted and purified peroxidases from their local radish, in UK (9), Turkey (2), Pakistan (10) and Japan (11) in order to find peroxidases with different specificity for promoting the development of new analytical methods and potential industrial processes (9).

The aim of this work was the extraction and partial purification of local radish peroxidase in order to find a locally available and economical source of peroxidase which can be used for practical application.

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Materials and Methods:-

Extraction of local radish roots:-

White radish roots were collected from local market, washed, minced and homogenized in blender 1:1 with 0.1M phosphate buffer pH (7.0), then the homogenate was filtered and centrifuged for 3000rpm/5min, 4°C the supernatant were collected and stored at -20°C until use (10).

Enzyme Assay and Protein Estimation:-

Peroxidase assay was carried out in a reaction mixture containing: 0.1M phosphate buffer (pH 7.0), 0.02M H₂O₂ (Substrate), 0.05M guaiacol (chromogen), and water (1:1:1:7). The absorbance of the colored complex was read on spectrophotometer at 420 nm wavelength after 30 sec of reaction interval (fig.1). One unit of activity was defined as the amount of enzyme that causes an increase of 0.001 absorbance per min (2). The protein concentration was determined by lowery method (12) using bovine serum albumin as a standard.

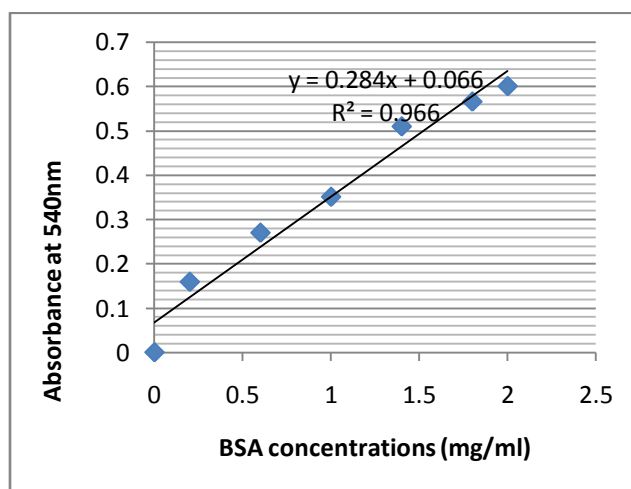


Fig 2:- Bovine serum albumin standard curve using lowery method.

Ammonium sulfate precipitation:-

Ammonium sulfate precipitation was carried out in homogenate on an ice bath for 20, 30, 40, 50, 60, 70 and 80% salt saturation. Ammonium sulfate was slowly added to the homogenate stirred until complete dissolution. Then the mixture was centrifuged at 3000 x g for 60 min, 4°C and the precipitate was dissolved in 2 ml of 25mM phosphate buffer (pH 7.5). The concentrated sample with maximum specific activity was selected and dialyzed in a dialysis tube three times for 2 days against 1 L above buffer at 4°C for further use (2).

Ion exchange chromatography:-

Ion exchange chromatography was done for dialyzed sample using DEAE-cellulose column (2 X 30 cm) equilibrated and washed with 25mM phosphate buffer (pH: 7.5) and eluted with [25mM potassium phosphate buffer (pH 7.0) buffer and 0-300mM KCl gradient], at a flow rate (3ml/5min) (10).

Results and Discussion:-

The enzyme was extracted by blending the radish roots with time intervals in order to avoid heating up the blended material. Blending is efficient method for breaking plant cells (10). Centrifugation for the blended material was done in order to remove particular materials and intact nuclei.

Several salt saturation percentages were used to precipitate the enzyme, the maximum activity was obtained with 80% salt saturation (fig.3). The fold of purification using ammonium sulfate was 15.28 times. Ammonium sulfate salt is widely used for protein precipitation because of its highly solubility which make the solutions have highly ionic strength (10). Several investigators precipitate peroxidase from their local radish using ammonium sulfate at 90% (13), 85% (10), and 60% (2) saturation. This differences in salt percentages used may be due to the different isozymes of peroxidase which precipitated at these percentages, since it was reported that seven isozymes were eluted from radish (13), the Korean radish roots contain six isozymes (14).

DEAE-cellulose chromatography was mostly used for peroxidase purification (10). Two fractions of peroxidase activity were eluted from DEAE-cellulose column (fig.4), the first was purified 35.62 times and showed a final specific activity 41.851U/mg with a 51.29% yield. The second was purified 23.45 folded and showed a final specific activity 27.551U/mg with a 42.12% yield. Other investigators were used DEAE-cellulose get 14.08 (10) and 9.7 (2) times of purification.

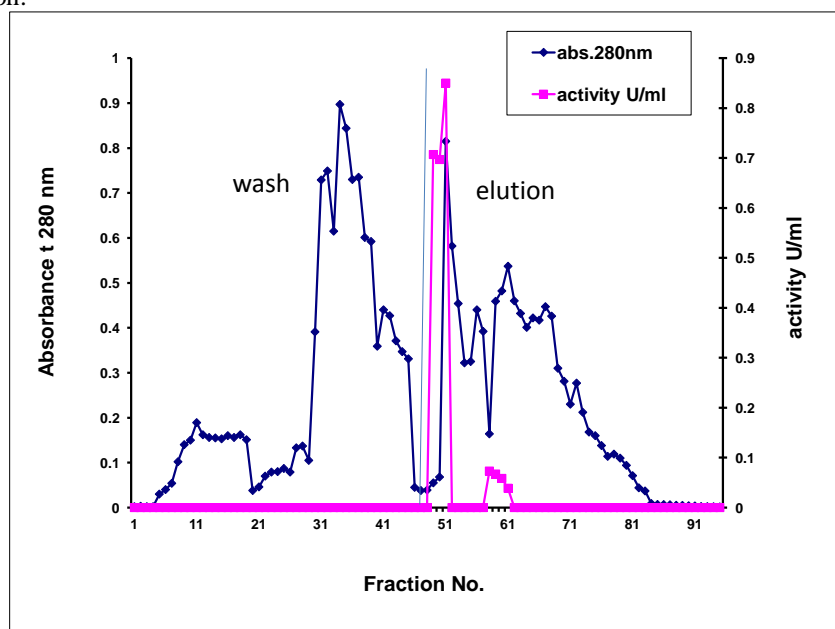


Fig 2:- Ion exchange chromatography for peroxidase extracted from radish roots. DEAE-cellulose column (2×30cm) equilibrated and washed with 25mM phosphate buffer (pH: 7.5) and eluted with [25mM potassium phosphate buffer (pH7.5) buffer and 0-300mM Kcl gradient], at a flow rate (3ml/5min).

Specific activity of POD in crude extract was 1.175 and increased during purification to 41.85 for the first eluted fraction and 27.55 for second (table1) from DEAE-cellulose column.

Table 1:- purification profile of peroxidase extracted from radish root

Purification step		Volume (ml)	Activity (U/ml)	Protein (mg/ml)	Specific activity (U/mg)	Total activity	Yield %	Fold of purification
Crude extract		25	0.141	0.120	1.175	3.525	1	1
Dialysis		10	0.295	0.038	7.763	2.950	83.86	6.61
DEAE-Cellulose Ion exchange chromatography	Peak1	8	0.226	0.0054	41.85	1.808	51.29	35.62
	Peak2	11	0.135	0.0049	27.55	1.485	42.12	23.54

The purification steps in this study seems to be suitable for fractionation of POD from Iraqi radish roots as the purification parameters showed (table1) in comparison with purification processes in other studies.

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