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Single step Affinity Purification and partial characterization of peroxidase from daikon (*Raphanus sativus*)

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Abstract: *Concanavalin A (con A) was immobilized on agarose beads and used as a ligand for affinity chromatography. The purification procedure was conduced by precipitation of the crude extract with 80% saturation ammonium sulfate followed by affinity chromatography. The purified daikon peroxidase contains carbohydrate moiety as revealed by staining the gels with Periodic Acid Schiff reagent (PAS staining). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (PAGE) yields one protein band. The molecular weight of purified protein is calculated by standard curve of reference proteins on gel after SDS-PAGE electrophoresis and it is found to be 45 KDa. The yield of the purified enzyme was about 24.5% with specific activity of about 500U|mg. The optimum pH and optimum temperature of the enzyme was found to be 5.0 and 40^o C respectively. The activity retained 100% of initial activity during 4 weeks of storage at 4^o C. Km of this enzyme was measured by Linewearver-Burk curve for guaiacol towards H_2O_2. For the purification from daikon a 24.5% recovery and a 142-fold enrichment was achieved. It suggests that Raphanus sativus is a good source of plant peroxidase, which could be used in manufacturing diagnostic diagnostic reagent in assays for glucose in place of commercially available horseradish peroxidase.*

Keywords: *Affinity chromatography, Agarose gel beads, Concanavalin A, Peroxidase.*

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I. Introduction

Peroxidases (EC: 1.11.1.7) belong to hemoprotein enzymes catalyzing the one electron oxidation of many organic and inorganic substrates using hydrogen peroxide oxygen as a hydrogen acceptor. These enzymes are widely distributed in a wide range of plant species (Van Huystee and Cairns, 1982) where they are involved in various processes e.g. cells in soluble, ionically or covalently bound to cell wall components forms (Keversand Gaspar, 1985), cell elongation by cell wall-bound peroxidase (Lee and Lin, 1995), lignification process (Cai et al., 2006) and plant defense mechanisms (Saravanakumar et al., 2007; Morkunas and Gmerek, 2007). Its properties and physiological roles in fruits and vegetables have been reviewed by several authors. Many studies have been done on amino-acid sequencing and heme structure of peroxidases [2, 3]. Several isoperoxidases, notably horseradish and turnip, have been studied in great detail during the past two decades [4].

Generally Enzymes are biological catalyst. Most of the enzymes accelerate biological reaction in the body [1]. From the economical point of view, peroxidase is an important enzyme. It has a wide range of application in health science, food industry and in diagnostic purposes as enzymatic determination of glucose, uric acid, cholesterol and many other metabolites in biological fluids and is also an important enzyme in ELISA systems [6]. It has been used in delignification of paper pulp. It is used in laundry detergents for bleaching of dyes in solution and thus preventing surplus dye from one garment to deposit on and decolorize other garments. Other potential applications are polymerization of chlorinated aromatic compounds in the treatment of waste-water, enhancement of flavors in food and polymerization of lignin in the production of various types of composite materials, where the use of artificial resins thus may be avoided [7]. Horseradish (*Armoracia rusticana* L.), the traditional source of peroxidases, does not grow well in Indian subcontinent. In our laboratory we are testing alternative economical sources more locally available and studying their properties as possible substitutes for horseradish peroxidase (HRP). Based on a previous screening experiment from our laboratory, it has been shown that tomato, sweet potato, cauliflower, cabbage, daikon etc. contain considerable amount of peroxidase. Among them, daikon contains the highest amount of peroxidase. The objective of this study is to extend our previous work towards the development of a new affinity chromatographic technique to isolate and purify peroxidase from daikon in a convenient way.

II. Materials and method

Preparation of agarose beads:

Uniform-sized agarose beads were prepared by emulsification technique where 4% w/v agarose was dissolved in water where a micro oven was used to heat. A mixture of liquid paraffin containing 2.5 wt% of Tween 80 emulsifier was used as oil phase at 80°C. The oil phase was rapidly mixed with water phase and agitated to obtain an emulsion. The emulsion was rapidly cooled bellow the gelation temperature by mixing with second liquid paraffin at –20°C under gentle agitation to form gel beads. After the gel beads rinse with water. Thus, Uniform sized agarose gel was prepared.

Preparation and modification of agarose beads via cross-linking followed by its treatment with epichlorohydrin:

About 50 ml uniform-sized agarose gels were taken in a 250 ml beaker containing 20 ml of 2 M NaOH and 5 ml of epichlorohydrin. The mixture was poured into two 50 ml falcon tube. The solutions containing gel were allowed to react for 2 hours at 40^{o}C with constant mixing in a home made rotary mixture to keep the concentration uniform. After reaction, the gels were washed with deionized distilled water until it became neutral.

Modification of agarose gel with 1,6-hexanediamine:

For the Modification of agarose gel, 300 ml 0.2 M 1, 6 hexadiamine solution containing 1.5 gm sodium carbonate decahydrate was mixed with the epoxy cross-linked agarose gels. The reaction was carried out at 60^{o}C for 2 hours. After reaction the gels were washed with distilled water until the gel became neutral [8].

Activation of agarose gel with 2,4,6-trichloro-1,3,5-trizine:

Amino modified agarose gel was soaked in 50ml 3M NaOH solution. It was placed in ice bath and 150ml of a 0.5M 2, 4, 6-trichloro-1, 3, 5-trizine acetone solution was mixed with home made mixture in refrigerator for 60 mins. After activation the gels were rinsed successively with 500ml 50 vol% acetone aqueous solution and 2 litter distilled water, to remove the unreacted 2, 4, 6-trichloro-1, 3, 5-trizine.

Immobilization of conA to the 2,4,6-trichloro-1,3,5-trizine activated gel:

2,4,6-trichloro-1, 3, 5-trizine activated gel was placed into an ice bath and mixed with 400ml of a 0.1M sodium phosphate, pH 7.8 buffer containing 400mg conA with the help of home made mixture in refrigerator for 12 hrs. After coupling, the gel was separated and 150 ml of a 0.5 M ethanolamine aqueous solution was mixed for 8 hrs to block the unreacted triazine groups. Finally the gels were rinsed with 1 litter distilled water [9].

Extraction of crude daikon extract:

The daikon was purchased from local vegetables market and washed thoroughly in distilled water. After thorough cleansing, the daikon was cut into small pieces as small as possible. The small pieces were blended with an electric blender. The blending was done for cell rapture and easy excretion of juice. The blender materials were collected in large beakers. The blending materials were poured into Tincture Press (HP-5, Karl Kolb, Germany). The juices were collected by compression using the Tincture Press at a maximum pressure of 400 bars. The juices were then filtered and centrifuged at 12,000 rpm using Ultra cooling centrifuge machine (3K-3000, Sigma, Germany) to remove any dirt material suspended in the juice. The supernatant was collected and dialyzed twice, for 12 h each time, against a 500 ml of the same buffer in a refrigerator. The obtained solution was filtered using a 0.1 μm microfiltration membrane. The clear supernatants were referred as crude extract.

Separation and purification of peroxidase using con A affinity matrix:

To pack the column, a slurry with binding buffer in a ratio of 75% settled medium to 25% buffer was prepared. All materials were equilibrated to the temperature at which the chromatography will be performed. The slurry was poured into the column in one continuous motion. To minimize the introduction of air bubbles, the slurry was poured down with glass rod held against the wall of the column. Immediately the remainder of the column was filled with buffer and the adjustable top cap of the column was mounted. Finally excess buffer inside the column was discarded by decreasing the volume of the column using adjustable top cap/piston and connected to a pump. The column was washed with 2 to 3 bed volumes of binding buffer in order to pack the bed and to equilibrate the column with buffer (20 mM Tris-HCl buffer pH 7.4 containing 0.5 M NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$ and 1 mM $MgCl_2$). A slightly higher flow rate can be used for packing than will be used for affinity chromatography. The crude sample of daikon was injected to the column equilibrated 20 mM Tris-HCl buffer pH 7.4 containing 0.5 M NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$ and 1 mM $MgCl_2$ at a flow rate of 0.5 ml/min. Washing of the column in order to remove unbound materials was carried out by pumping 20 mM Tris-HCl buffer pH 7.4 containing 0.5 M NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$ and 1 mM $MgCl_2$ at a flow rate of 1 ml/min until the absorption at 280nm in HPLC detector became linear. After washing, the bound protein was eluted with elution buffer (20 mM Tris-HCl buffer pH 7.4 containing 0.4 M glucose). The flow rate of the mobile phase was 1ml/min. The chromatographic purification was carried out as the separation procedure.

Determination of the protein content by Biuret method:

Reagent preparation: The reagent was prepared by mixing the following solution.

Sodium hydroxide	**200 mM**
Potassium sodium tartrate	**32 mM**
Copper sulfate	**12 mM**
Potassium iodide	**30 mM**

Standard: Standard contains protein 80 gm/l and sodium azide 0.095%.

20 µl protein sample or 20 µl standard protein (Bovine Serum Albumin) sample were added to 1.0 ml of total protein reagent in different test tube, the mixture was shaken for few times, allowed to react for 10 min at room temperature. The absorbance of sample and standard against the reagent blank were measured within 30 min at 546 nm using a UV-Vis Spectrophotometer. The amount of protein content (crude extract and purified protein) was determined using the following equation.

$$C = 80 \times \frac{\Delta A_{sample}}{\Delta A_{standard}} [mg/ml]$$

Determination of the activity of the peroxidase:

Based on that Bergmeyer the activity of the peroxidase was determined using hydrogen peroxide as substrate. To prepare the substrate solution, 0.1 ml 30% hydrogen peroxide was diluted with distilled water to 120 ml and adjusted A_{240} in 1 cm light path to 0.4 - 0.41. Guaiacol is a color forming dye prepared by 18 mM Guaiacol solution. To determine the enzyme activity a buffer solution (25 mM phosphate buffer, pH 7.0 containing 500 mM NaCl, 1 mM $MnCl_2$ and 1mM $CaCl_2$) contains with substrate and guaiacol. To ensure accuracy, the substrate solution was prepared daily. For the determination of the peroxidase, a suitable amount of enzyme solution was added to 50 µl of the above solution, and the absorbance at 436 nm was monitored by measuring the rate of color development spectrophotometrically 25^0 C. The concentration was calculated by using for the absorption coefficient the value $\varepsilon=3.9\times10^4$ M^{-1} cm^{-1}.

Specificity: One of the properties of enzymes that makes them as important as diagnostic and research tools is the specificity they exhibit relative to the reactions they catalyze. For many enzymes the correct three dimensional structures are essential to function. Failure to fold into the intended shape usually produces inactive enzymes with different properties. Several neurodegenerative and other diseases are believed to result from the accumulation of misfolded (incorrectly folded) enzymes [10].

Enzymes are usually very specific as to which reactions they catalyze and the substrates that are involved in these reactions. Complementary shape, charge and hydrophilic/hydrophobic characteristics of enzymes and substrates are responsible for this specificity. peroxidase can also show impressive levels of stereospecificity, regioselectivity and chemoselectivity [11].

Reagent preparation and enzymatic Determination of glucose:

Glucose was determined enzymatically by an assay reaction mixture containing 0.1 M phosphate buffer, pH: 7.5, 0.75 mM phenol, 0.25 mM aminoantipyrine, 15 ku/l glucosel oxidase, 2 ku/l Mutarotase, and 1.5 ku/l purified peroxidase enzyme of daikon. The reaction was started by the addition of 25 µl of glucose standard 100mg\dl. The changes in absorbance were measured at 546 nm after 10 min incubation at 37°C against a blank containing 25 µl of water instead of glucose.

III. Results and discussion

Lectin-afinity chromatography has been widely used for the puriication and analysis of oligosaccharides, glycopeptides, and glycoproteins. However, most of the lectin-afinity columns currently available are agarose bead-based columns. In order to increase the mechanical strength of agrose gel, epichlorohydrin was used as crosslinker. Hexadiamine was used as spacer arm. Finally affinity ligand conA was coupled to the spacer arm using amino reactive bi-functional crosslinker, trizine. With the growing interest in glycoproteomic studies, such as biomarker identiication, there is an increasing need for a robust HPLC lectin column. Presented here is the development and applications of a new monolithic Concanavalin A (Con A) afinity column. Concanavalin A (28kDa) is a lectin derived from *Canavalia gladiata* (sword bean) seeds. Con A is one of the most well characterized and widely used lectins. It binds to α-mannose, and to α-glucose with weaker afinity. Divalent metal ions such as calcium (Ca2+) or magnesium (Mg2+) need to be present to keep Con A active for its binding to carbohydrates. Finally affinity ligand conA was coupled to the spacer arm using amino reactive bi-functional crosslinker, trizine. The HPLC compatibility of this column allows fast and eficient

puriication and analysis for various Con A-binding glycoconjugate samples. When the crude extract was passed through the prepared matrix gel, only the peroxidase binds to the gel matrix and the remaining other protein was passed as flow through. When a molecule which has higher affinity for conA than peroxidase binds to conA matrix and replaces the peroxidase. To elute the peroxidase the concentrated glucose solution was used.

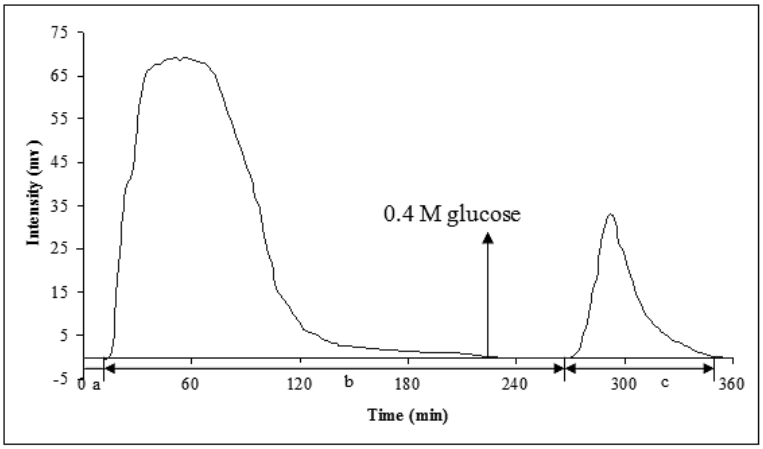


Figure 1: Chromatogram of affinity purification, (a) Equilibrate with 20 mM Tris-HCl, pH 7.4 containing 0.5 M NaCl, 1 mM MnCl2 and 1 mM CaCl2; (b) Washing with 20 mM Tris-HCl, pH 7.4 containing 0.5 M NaCl, 1 mM MnCl2 and 1 mM CaCl2; (c) Elution of ConA binding protein with 20 mM Tris-HCl, pH 7.4 containing 0.4 M glucose.

Detection of carbohydrate in peroxidase:

The presence of sugar as well as total neutral sugar content of the peroxidase was detected by phenol sulfuric acid method (Dubois et al1956) and staining the gels with Periodic Acid Schiff reagent (PAS staining), as describedin the Pharmacia Manual (Polyacrylamide Gel Electrophoresis Laboratory Techniques, revised edition, 1983). PAS staining on gel electrophoresis was used to identify individual sugar molecules present at various stages of the protein purification. A= Crude extract, B= Flow through and C= peroxidase purified protein sugar

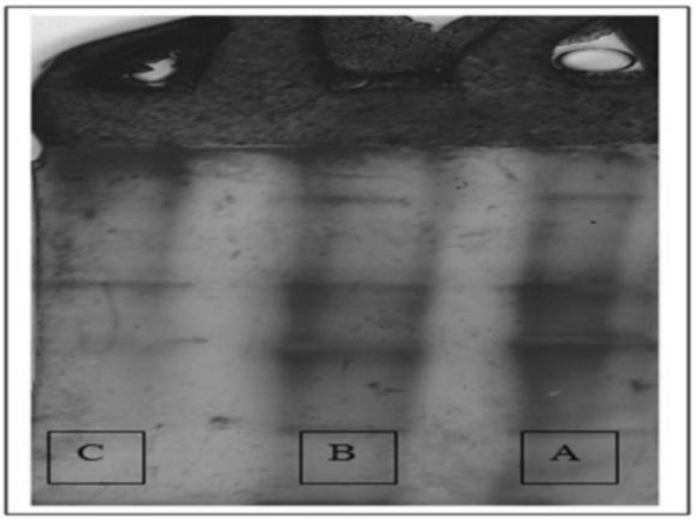


Figure: PAS staining on gel electrophoresis. A sugar analysis revealed peroxidase is a glycoprotein since it stained purplish pink with Schiffs reagent after PAGE. A single purplish pink band indicated the sugar molecules present the purified protein

UV-Vis spectrum of peroxidase at different stage of purification:

A protein consists of an amide (peptide) backbone with various side chains on the α carbons between each amide. The dominant chromophore is the amide group, which has a weak $n\pi^*$ transition at about 220 nm and an intense $\pi\pi^*$ transition at about 195 nm. The elecronic transitions of most side chains occur bellow 200 nm, and

are overpowered by intense ππ* of the amides. Exceptions are phenylalanine, tyrosine, tryptophan, cysteine, methionine, and disulfide groups, which begin their electronic transitions just below 300 nm. The π systems of phenylalanine, tyrosine, and tryptophan have the ππ* transitions. . The spectrum was taken in 20 mM Tris-HCl buffer, pH 7.4. We are primarily interested in the spectra of affinity purified protein and the result has been presented in figure 11.

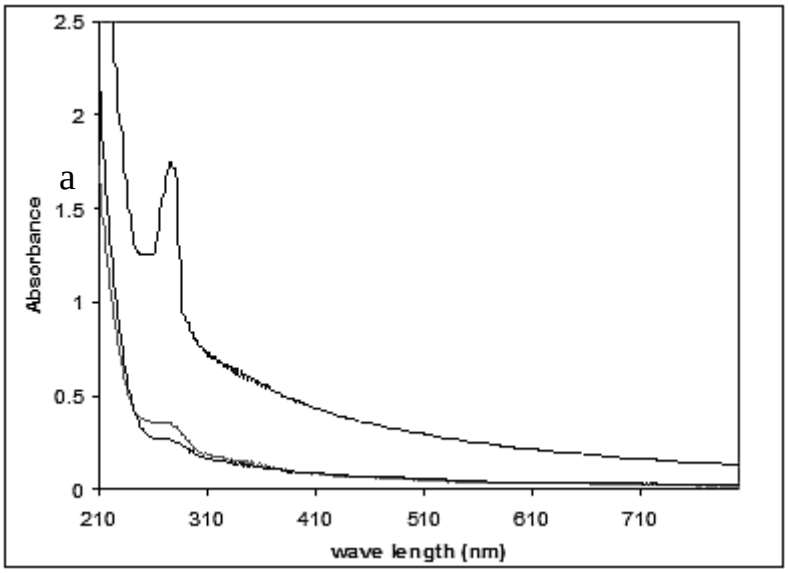


Figure 11: UV-Vis spectrum of (a) Crude extract, (b) Flow through (unbound) protein, and (c) Elution (bound) protein.

Determination of the protein content by Biuret method:

Protein content of the peroxidase sources was determined by above procedure and the results have been presented in table 2. It was observed that two pieces of daikon contains about 1.024 gm of protein among which about 0.264 gm is conA-binding protein and the remaining 0.731 gm is other protein. Thus, the approximate ratio of conA-binding protein and other proteins in daikon is 1:3

Table 2: A summary of the purification of peroxidase from daikon by home-made affinity chromatographic technique.

	Volume (ml)	Absorbance at 546 nm	Protein content (mg/ml)	Total protein (mg)
Standard		0.41	80	
Crude extract	150	0.035	6.82	1024
Unbound	150	0.025	4.88	731.7
Bound	30	0.045	8.8	264

Test of purity by SDS-PAGE:

Purity test of peroxidase by SDS-PAGE:

Purity of the bound fractions from the affinity columns was checked by SDS-PAGE analysis, and was performed under reducing conditions, on a 1 mm separating gel in a final concentration of 12.5% of acrylamide-bisacrylamide solution. About 10 µg of total proteins were analyzed by performing the electrophoresis. Detection of the protein bands was performed with the Coomassie Brilliant Blue R-250 staining method, and purity degree was determined by electronic scanning. The scanning result has been presented in figure 15. In figure, lane B indicating the presence of six kinds of protein in crude extract. However, the presence of single band as shown in lane D indicating that our affinity matrix system was able to purify only one kind of protein in a single step. Thus, our affinity matrix system was proved to be a useful tool in the purification of ConA immobilized binding protein in a single step.

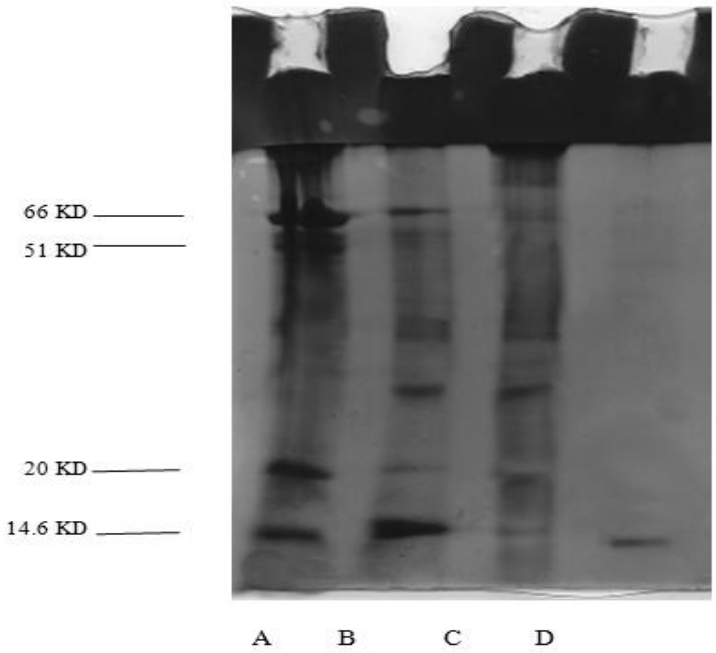


Figure 15: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to identify individual proteins present at various stages of the purification. A= Marker protein, B = Crude extract, C= Flow through and D= Affinity purified protein.

Determination of molecular weight of Peroxidase by SDS-PAGE:

The molecular weight of the affinity purified peroxidase was determined by SDS-PAGE using Bovine Serum albumin (66 KDa), Fetuin (51 KDa), Trypsin inhibitor (20 KDa) and Lysozyme (14.6 KDa) as reference protein. The molecular weight was calculated from the standard curve of reference proteins which was constructed by plotting $\log_{10}$ molecular weight against relative mobility of the proteins on gel after electrophoresis (Figure 16) and the molecular weight of the purified protein was found to be 14 KDa.

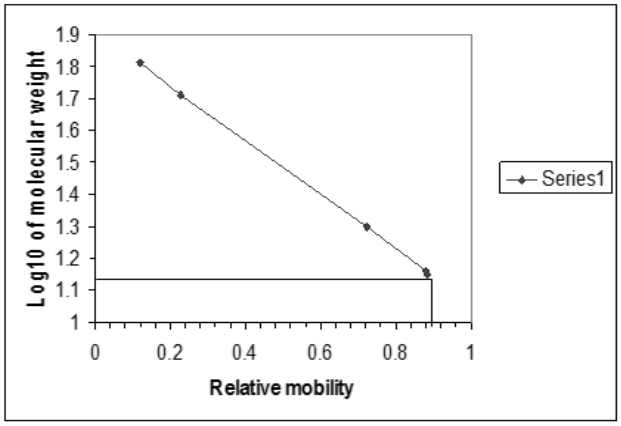


Figure 16: Estimation of the molecular weight of the purified peroxidase derived from daikon. Relative mobilities of marker proteins (labeled l–4) were used to obtain the standard curve as described by Shapiro et al. and Weber and Osborn [23, 24]. 1, 2, 3, and 4 were standard markers of mol wt 66 KDa, 51KDa, 20 KDa and 14.6 KDa, respectively.

Optimum temperature and thermal stability:

Thermal stability of daikon peroxidase (900 u/ml) at temperatures ranging from 30-60°C after 30 min was also measured. As it is shown in Figure, daikon peroxidase keeps more than 90% of its activity up to 40°C, but in higher temperatures it loses most of its activity. At optimum pH (pH: 5) daikon peroxidase exhibits the typical linear Linewearver-Burk plot. Km and Vmax value 5 for daikon peroxidase are about 0.56×10−3 M and 7.3×10−3 M/min respectively.

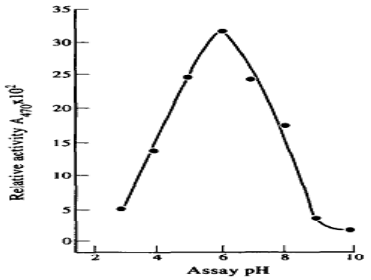
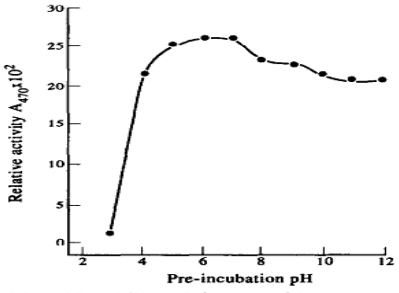


Fig. 6. The optimum pH of the peroxidase.



The pH stability of the peroxidase.

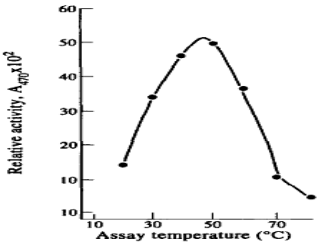


Fig. 4. The optimum temperature of the peroxidase assay.

Optimum pH and stability of the peroxidase:

pH stability of daikon peroxidase in 0.02 M tris -HCl buffers with pHs between 4-8. After four hours of incubation of daikon peroxidase (150 u/ml) in mentioned pHs at 40°C, the activity was assayed. The maximum stability for daikon peroxidase after four hours is in pH: 5. In pHs ranging 5.5-8, daikon peroxidase loses only 20% of its total activity. In higher pHs than 7.5 it loses more than 60-80% of its total activity.

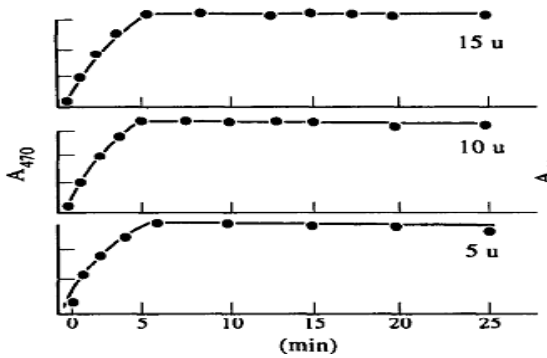
Storage stability of the peroxidase:

When stored at 4^0C in 7.0, daikon peroxidase was found to be very stable. They retained 100% of initial activity during 4 weeks of storage; afterwards their activity gradually decreased (after 6 weeks they retained about 65% of their initial activities and 12 weeks they retained about 6% of their initial activities). The daikon peroxidase stability was shown below in fig

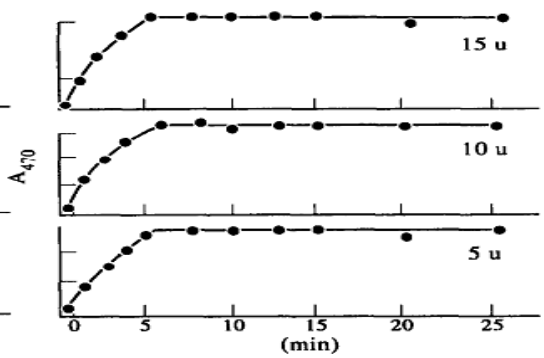
Km Determination:

Michaelis-Menton constant for the peroxidase towards substrate guaiacol and H_2O_2 was determined by incubating daikon peroxidase (150 u/ml) during one minute with varying concentrations of H_2O_2 and fixed saturation concentration of guaiacol, then for the best conclusion, we used Lineweaver-Burk plots to determine Vmax and Km

The purified daikon peroxidase was assessed in assays for glucose in comparison with a commercially available horseradish peroxidase for Glucose determination. As shown in Fig. daikon peroxidase showed similar reaction curves to those of the commercially available product at the levels of activity examined both in respect of the time taken to reach a plateau of absorbance at 546 nm, and the maximum absorbance achieved.



(a)Daikon peroxidase



(a) Horseradish peroxidase

Fig. Daikon peroxidase was used as a diagnostic reagent: 5, 10 and 15 units of the prepared kits (a), or of a commercial available glucose test kits from crescent (b), were used in each test for the rate assay of glucose. Performance of the two sources of enzvme were compared in terms of the time (min) to reach a plateau absorbance at 546 nm and the maximum absorbance achieved at the end of the reaction.

The results were also in agreement with the results of kits prepared with horseradish peroxidase with glucose test kits from crescent, and in fact there was no significant different between results. Moreover the blank solution containing all reagents except glucose is colorless and quite clear. The rate of reaction, in presence of glucose and 546 nm was equal to glucose test kits from crescent. Prepared kits or glucose test kits from crescent was found to be as efficient as each other.

Table 3. Separation of peroxidase from horseradish by affinity matrix.

	Volume of the sample (ml)	Total protein content (mg)	Total peroxidase activity (U)	Yield (%)	Purification (fold)
Initial extract sample	100	156.3	1.44×10^7		
Flow through	100	126.5	1.42×10^7	99	1.2
Purified protein	40	0.52	0.35×10^7	24.5	142

The specific activity of the product can be calculated by dividing the activity with the protein content, and the protein content was determined using the Coomassie Brilliant Blue method. Several authors have studied on peroxidase. Schoenbein (1855) was the first who searched vigorously on peroxidase [12]. Peroxidase was the first one isolated and investigated in 1936 by summer and Howell [13]. Peroxidases have been purified from such diverse sources as horseradish, yeast, sweet potato, turnip, and wheat during 1942-1956 [14]. One of the first successful methods in purification and crystallization of purified peroxidase was performed by Shannon and his colleagues in 1966 [5]. He separated about 7 isoenzymes, from horseradish by ammonium sulfate precipitation and using ion-exchange chromatography. Delincee reported about 20 peroxidase isoenzymes by thin layer isoelectric focusing [15]. In 1968, Mazza and his colleagues worked on purification of turnip peroxidases and they separated about seven isoperoxidases [16]. Ion exchange and affinity chromatography have already been used for purification of peroxidase from horseradish [17]. In this work, after fractionation by acetone, main cabbage peroxidase (BOC-POD) was successfully purified by ion exchange and affinity chromatography. Comparing with crude extract, the recovery of purified enzyme was not very high, but purity, as was shown by SDS-PAGE analysis and RZ, was very high. Therefore this method may be useful for purification of peroxidase from sources with high protein content. It has been reported that peroxidase isoenzymes from various sources have different molecular weights ranging from 30,000-60,000 Dalton [18], e.g. four isoenzymes of turnip peroxidase have molecular weights between 37,000 and 57,000 Dalton and 30,000-54,000 for Japanese radish isoperoxidases [16]. The molecular weight (MW) of daikon peroxidase was about 45,000 Dalton, which is in the range of MW of most the known peroxidase. Peroxidases are mainly used in determination of metabolites with other enzymes; therefore it is required to have good thermal stability and activity in a wide range of pH. daikon peroxidase is more active and stable in acid pHs. Horseradish peroxidase is more active and stable in neutral pHs and therefore daikon peroxidase could be a suitable alternative whenever more stability and activity in acidic conditions are needed. Optimum pH and temperature for cabbage peroxidase and its stability are comparable with other reports too [19-21]. For example, optimum pH and temperature for strawberry peroxidase are reported to be 6 and 30°C respectively. This enzyme keeps almost its original activity after heat treatment up to 40°C for 20 min at pH: 6.0-8.0 (22). Comparing Km values of cabbage peroxidase with six isoenzymes of Korean radish shows that Cabbage has 0.5-1 times Km values comparing with Korean ones towards O-dianisidine (4). Daikon peroxidase is a vegetable available almost in all seasons at very low price and large quantity in our country. Although peroxidase content of daikon might not to be as high as horseradish peroxidase, most of its properties are similar to HRP and by this procedure it is possible to obtain highly purified peroxidase, suitable for diagnostic application.

IV. Conclusions

In conclusion Daikon(*Raphanus sativus*). peroxidase has been successfully purified by the research. The satisfactory recovery of activity and RZ as well as the simplicity of the procedure make this strategy a low cost alternative for the purification daikon(*Raphanus sativus*). Peroxidase.

References

[1]. AD. Smith (Ed), et al. (1997) Oxford Dictionary of Biochemistry and Molecular Biology. pp. 43, ISBN 0-19 854768-4.
[2]. Welinder K.G. and Mazza G. Amino acid sequences of heme-linked, histidine-containing peptide of five peroxidases from horseradish and turnip. Eur. J. Biochem., 73: 353-358 (1977).
[3]. Welinder K.G. and Mazza G. Covalent structure of turnip peroxidase 7. Ibid, 108: 481-489 (1980).
[4]. Young L.M. and Kim S.S. Characteristics of six peroxidases from Korean radish root. Phytochemistry, 35: 287-290 (1994).
[5]. Shannon L.M., Kay E. and Lew J.Y. Peroxidase isoenzymes from horseradish roots: I. Isolation and physical properties. The J. of Biological Chemistry, 241:
[6]. 2166-2172 (1965).
[7]. Charmaine Solomon, Periplus, (1998) Encyclopedia of Asian Food.
[8]. Cuatrecasas, P. (1970) Agarose derivatives for purification of protein by affinity chromatography. Nature 228; 1327.
[9]. Wei Guo, Eli Ruckenstein. (2002). 2001;Crosslinked mercerized cellulose membranes for the affinity chromatography of papain inhibitors. Journal of Membrane Science; 197:53-62.
[10]. Wei Guo, Eli Ruckenstein.(2003). Separation and purification of
[11]. Dennis J. Selkoe (2003). Folding proteins in fatal ways. Nature 426 (6968): 900–904.
[12]. KE. Jaeger, T. Eggert. (2004), Curr Opin Biotechnol, 15(4), 305–313.
[13]. 12. Boyer D.B., Hardy H. and Myrbarck K. The Enzymes. Vol. 8, Paul K.G. Peroxides, Academic Press, 227- 275 (1963).
[14]. Mahaly A.C. Plant Peroxidase in :Methodes in Enzymology. Academic Press, II: 801-803 (1995).
[15]. Klapper M.H. and Hackett D.P. Investigation on the multiple components of commercial horseradish peroxidase. Biochem. Biophys. Acta, 96: 272-278 (1965).
[16]. Delincee H. and Radola B.J. Thin layer isoelectric focusing on sephadex layers of horseradish peroxidase. Biochemica Et Biophysica Acta, 200: 404-407 (1970).
[17]. Mazza G., Job C. and Bouchet M. Chemical composition and hydrodynamic characteristics of turnip peroxidases.Ibid, 322: 218-223 (1973).
[18]. Wagner M. Purification of HRP, using affinity chromatography on concunavalin-A agarose. Acta Biol. Med. Ger., 39: 1429-1431 (1975).
[19]. Kim S.S., Wender S.H. and Smith E.C. Isolation and Characterization of two peroxidases from Tobacco tissue cultures. Phytochemistry, 19: 165-168 (1980).
[20]. Rhee H., Park K.S., Cha Y.S. and Lee S.Y. Purification
[21]. and characterization of peroxidase from Chinese cabbage. Sanop Misaengmul Hakhoechi, 19: 470-476 (1991).
[22]. Jiee W.J., Cho N.S., Kim I.C., Park K.H. and Choi E.H.
[23]. Isolation and characterization of Fuji apple peroxidase. Hanguk Sikum Kwahakhoechi, 23: 442-446 (1991).
[24]. Kang S.O., Shiw K.S., Han Y.H., Young H.D. and Hah Y.C. Purification and characterization of an extracellular peroxidase from white-root fungus Pleurotus ostreatus. Biochem. Biophys. Acta, 1163: 158-164 (1993).
[25]. Civello P.M., Martinez G.A., Chares A.R. and Anon M.C. Peroxidase from strawberry fruit (Fragaria anannassa Ducd.): Partial purification and determination of some properties. J. Agric. Food. Chem., 43: 2596-2601 (1995).
[26]. Shapiro, A. L., Viñuela, E. and Maizel, J. V. Jr. (1967) Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. Biochem. Biophys. Res. Commun. 28; 815-820.
[27]. Weber, K. and Osborn, M. (1969). The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis, J. Biol. Chem. 244; 4406–4412.

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