Research Paper

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Partial Purification of Peroxidase from Tomato

M. Anjum Zia, Khalil-ur-Rehman, M. Khalid Saeed,
Aftab Ahmed and Abdul Ghaffar

Peroxidase from tomato was extracted and partially purified by means of ammonium sulphate [$(NH_4)_2SO_4$] precipitation technique and ion exchange chromatography. The crude extract having the specific activity of 0.239U mg^{-1} was subjected to $(NH_4)_2SO_4$ precipitation technique for partial purification of peroxidase. The specific activity of $(NH_4)_2SO_4$ precipitated enzyme was 0.546U mg^{-1} with protein contents of 0.412mg ml^{-1}. After ion exchange chromatography through DEAE-Cellulose columns, the specific activity was 11.844U mg^{-1} and the protein contents were decreased to 0.0358mg ml^{-1} showing that unnecessary proteins have been eliminated. Finally, partially purified enzyme was subjected to SDS-Electrophoresis for confirming the purification. So it was concluded that crude and partially purified enzymes from tomato possess an appreciable enzyme activity.

Key words: Purification, peroxidase, tomato, electrophoresis

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For further information about this article or if you need reprints, please contact:

M. Anjum Zia
Department of Chemistry (Biochemistry),
University of Agriculture,
Faisalabad, Pakistan

E-mail: anjum_ranapk@yahoo.com

ANSInet
Asian Network for Scientific Information

Department of Chemistry (Biochemistry), University of Agriculture, Faisalabad, Pakistan

Introduction

Peroxidase (E.C. 1.11.1.77) belongs to the class oxidoreductase, is an iron-porphyrin ring containing enzyme that catalyzes the redox reaction between hydrogen peroxide (H_2O_2) as an electron acceptor and substrates by means of O_2 liberation (Brill, 1996). The enzyme is present naturally in plants e.g., soybean, radish, horseradish, tomato, potato, carrot, turnip, wheat, dates, beats and strawberry etc. (Reed, 1975; Ambreen *et al*., 2000).

Peroxidase has wide applications in health sciences as a diagnostic tool. Autoantibodies directed against the thyroid peroxidase are widely used to diagnose human autoimmune thyroid disease (Nord, 1953). A variety of enzymes including peroxidase, alkaline phosphatase, urease and β-D-galactosidase etc. have been used in ELISA kits. Among which peroxidase is widely used to prepare "antibody-enzyme" or "anti-antibody-enzyme conjugates" for ELISA due to its high turn over rate, rapid availability, ease of conjugation and better sensitivity (Kemeny and Challacombe, 1989; Zia *et al*., 2000).

Nevertheless, no remarkable work on this enzyme has so far been done in Pakistan. The commercial peroxidase is imported for use either by quality control laboratories of food, pharmaceutical industry and enzymological research purposes. Therefore, this investigation comprises the isolation and extraction of peroxidase from tomato and purification by $(NH_4)_2SO_4$ precipitation technique, DEAE-Cellulose chromatography and electrophoresis.

Materials and Methods

Extraction: 100gm of tomato were added to 400ml dist. water and blended thoroughly for 15 min. Contents were centrifuged at 10,000 rpm for 15 min., and the supernatants were heated at 65°C for 3 min. in a water bath to inactivate catalase present in extract (Rehman *et al*., 1999; Ambreen *et al*., 2000).

Purification of Peroxidase: The enzyme extract was subjected to $(NH_4)_2SO_4$ precipitation technique (Jen *et al*., 1980) for partial purification. The column of DEAE-Cellulose was prepared by the method of Cooper (1977). Exchanger was rapidly swollen by heating the slurry at 90°C for 5 hrs., using a water bath. Slurry was poured into the column to fill completely the required column bed height. After the column packing, then a layer of sample was applied on its top with the help of a pipette. The 25 fractions of 2ml of eluent were collected at constant drop rate which were subjected to enzyme assay.

Enzyme Assay and Protein Estimation: Phosphate buffer of pH 6.5 containing 0.320ml of H_2O_2 and 2ml guaiacol as chromogen were used, and optical density (OD) was noted at 470nm wavelength after 3 min of reaction interval for enzyme assay (Ambreen *et al*., 2000). Protein contents of the enzyme extract at various steps were measured by biuret method (Gornall *et al*., 1949; Zia *et al*., 2000).

SDS Electrophoresis: Polyacrylamide gels were prepared with SDS and sample was employed to electrophoresis according to the method of Habib (1999). The molecular weight of the enzyme was determined by the method of Laemmli (1970).

Results and Discussion

Enzyme was extracted by blending the tomato for 15 min. with short intermissions. The intermissions were to avoid heating up of blended material. The enzyme and specific activity of crude extract obtained were 0.118U ml^{-1} and 0.239U mg^{-1}, respectively (Table1).

Table 1: Purification of tomato peroxidase

Type	Enzyme activity U ml^{-1}	Specific activity U mg^{-1}	Protein contents mg ml^{-1}
Crude extract	0.118	0.239	0.494
$(NH_4)_2SO_4$	0.225	0.546	0.412
DEAE-Cellulose	0.424	11.844	0.0358

DEAE- Cellulose ; Diethyl amino ethyl cellulose
$(NH_4)_2SO_4$; Ammonium sulfate

To purify the desired enzyme, extract was subjected to 50-90% saturation with $(NH_4)_2SO_4$. It is the most commonly used reagent for salting out of the protein because of its high solubility permits the achievement of solution with high ionic strength (Voet *et al*., 1999). Evans (1968) standardized the saturation values of $(NH_4)_2SO_4$ as 50-85% while Rehman *et al*. (1999) purified peroxidase from different seeds with 35-90% precipitation. This difference may be due to the concentration of salt used and duration of saturation for purification. The results are presented in Table 1 which are in line with Jen *et al*. (1980) who purified peroxidase from tomato. Ambreen *et al*. (2000) reported that specific activity of soybean peroxidase is 0.865U mg^{-1} after $(NH_4)_2SO_4$ treatment. The results of this investigation are somewhat different with that of Rehman *et al*. (1999) who purified peroxidase from horseradish but these are due to the difference of source selected for enzyme extraction and purification. Peroxidase exist in many isozymic forms, majority of which are anionic (Evans, 1968). The most often used cellulosic anion exchanger is Diethyl amino ethyl cellulose (DEAE- Cellulose) (Voet *et al*. 1999; Rehman *et al*. 1999). The specific activity obtained was 11.844U mg^{-1} after such treatment which indicates a high degree of purity as compared to prior method (Table1). Rehman *et al*. (1999) repotted that specific activity is 15.210U mg^{-1} of horseradish peroxidase. The findings of this research work was similar with Jen *et al*. (1980) who also reported that specific activity increased after DEAE chromatography treatment as compared to $(NH_4)_2SO_4$ treatment. So, it is concluded that tomato is also a rich source of this enzyme as well as horseradish.

To seek the purity of tomato peroxidase, SDS-PAGE was employed on the purified enzyme in horizontal gel. In this case, only one band was observed that confirmed the enzyme purity. Molecular weight estimation by SDS-Electrophoresis showed our preparation to be of 40,000 Dalton. As reported by Jen *et al*. (1980) that of 43,000±2000 Dalton and same also by Kokkinakis and Brooks (1979). The absorbance of enzyme extracts were recorded at 420nm after 3 min interval of reaction period. It was observed that there is a gradual increase in the activity of enzyme with increase in duration of time period. These results are in accordance with Rehman *et al*. (1999) and Ambreen *et al*. (2000) who found that a constant trend of increase in absorbance values with the increasing time period. Guaiacol was used as a chromogen in this investigation which has the high affinity for the enzyme substrate H_2O_2 to produce a color product.

In addition to this work; we have had good success using these techniques for the purification of horseradish peroxidase (Rehman *et al*., 1999) and soybean peroxidase (Habib, 1999; Ambreen *et al*. (2000). Similar methods, have developed for the purification of glucose oxidase and mutarotase to produce and standardize glucose estimation kit and such procedures can be developed to isolate and purify the other beneficial enzymes in more homogenous states. In brief, it is concluded

that tomato is a good source of peroxidase enzyme whose activity and production on commercial scale can be enhanced by improved purification techniques.

References

Ambreen, S., K. Rehman, M. A. Zia and F. Habib, 2000. Kinetic studies and partial purification of peroxidase in soybean. Pak. J. Agric. Sci., 37: 119-122.

Brill, A. S., 1996. Peroxidase and catalase comprehensive Biochemistry. Vol. XIV, Elsevier Pub. Co. Amsterdam., pp: 447-479.

Cooper, J. G., 1977. Tools of Biochemistry. Univ. of Pitts-Brough, USA., pp: 50-54.

Evans, J. J., 1968. Peroxidase from the extreme dwarf tomato plant: identification, isolation and partial purification. Plant Physiol., 43: 1037-1041.

Gornall, A. G., G. Bardwill and M. M. David, 1949. Determination of serum proteins by means of biuret reagent. J. Biol. Chem., 177: 751-766.

Habib, F., 1999. Partial purification of peroxidase from soybean. M. Sc. thesis, Dept. of Biochemistry, University of Agriculture, Faisalabad, Pakistan.

Jen, J. J., A. So and W. H. Flurkey, 1980. Tomato peroxidase: purification via hydrophobic chromatography. J. Food Sci., 45: 60-63.

Kemeny, D. M. and S. J. Challacombe, 1989. ELISA and other solid phase immunoassays. John Wiley and sons. USA., pp: 1-16.

Kokkinakis, D. M. and J. L. Brooks, 1979. Tomato peroxidase purification, characterization and catalytic properties. Plant Physiol.

Laemmli, U. K., 1970. Cleavage of structural proteins during the assembly of the hand of bacteriophage T4. Nature, 222: 680.

Nord, F. F., 1953. Advances in enzymology and related subjects of biochemistry. Interscience Pub., 15:156-158.

Reed, G., 1975. Oxidoreductase, In: enzymes in food processing. Academic Press, USA., pp: 216.

Rehman, K., M. Yaqub, M. A. Sheikh and M. Arshad, 1999. Extraction and evaluation of peroxidases from various vegetable sources. Int. J. Agric. Biol., 13:170-73.

Voet, D., J. G. Voet and C. W. Pratt, 1999. Fundamentals of Biochemistry. John Wiley and sons, USA., pp: 99-105.

Zia, M. A., M. Yaqub, K. Rehman and T. Mahmood, 2000. Production of rabbit antibuffalo antibodies horseradish peroxidase conjugate and standardization of ELISA for *Pasteurella multocida* antibodies. Pak. J. Biol. Sci., 3: 1716-1718.

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