Partial Purification of Peroxidase from Tomato

M. Anjum Zia, Khaliq-ul-Rehman, M. Khalid Saeed, Aftab Ahmed and Abdul Ghaffar

Peroxidase from tomato was extracted and partially purified by means of ammonium sulphate [(NH₄)₂SO₄] precipitation technique and ion exchange chromatography. The crude extract having the specific activity of 0.239U mg⁻¹ was subjected to (NH₄)₂SO₄ precipitation technique for partial purification of peroxidase. The specific activity of (NH₄)₂SO₄ 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
Introduction

Peroxidase (EC. 1.11.1.77) belongs to the class oxidoreductase, is an iron-porphyrin ring containing enzyme that catalyzes the redox reaction between hydrogen peroxide \( \text{H}_2\text{O}_2 \) as an electron acceptor and substrates by means of \( \text{O}_2 \) liberation (Brill, 1959). The enzyme is present naturally in plants e.g., soybean, radish, horseradish, tomato, potato, carrot, turnip, wheat, dates, beets 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 \( \beta \)-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 (Kemény 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 \( \text{(NH}_4\text{)}_2\text{SO}_4 \) precipitation technique, DEAE-Cellulose chromatography and electrophoresis.

Materials and Methods

**Extraction:** 100g of tomato were added to 400ml distilled water and blended thoroughly for 15 min. Contents were centrifuged at 10,000 rpm for 15 min., and the supernatants were heated at 85°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 \( \text{(NH}_4\text{)}_2\text{SO}_4 \) precipitation technique (Jan 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 50°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 \( \text{H}_2\text{O}_2 \) and 2ml guaiacol as chromogenic 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 intermittences. The intermittences 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 (Table 1).

<table>
<thead>
<tr>
<th>Table 1: Purification of tomato peroxidase</th>
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<tr>
<td>Type</td>
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<td>Crude extract</td>
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<td>( \text{(NH}_4\text{)}_2\text{SO}_4 )</td>
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<td>DEAE-Cellulose</td>
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To purify the desired enzyme, extract was subjected to 50-90% saturation with \( \text{(NH}_4\text{)}_2\text{SO}_4 \). 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., 1989). Evans (1990) standardized the saturation values of \( \text{(NH}_4\text{)}_2\text{SO}_4 \) 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 Jan et al. (1980) who purified peroxidase from tomato. Ambreen et al. (2000) reported that specific activity of soybean peroxidase is 0.885U mg\(^{-1}\) after \( \text{(NH}_4\text{)}_2\text{SO}_4 \) 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 cellulolic 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 (Table 1). Rehman et al. (1999) reported that specific activity is 15.210U mg\(^{-1}\) of horseradish peroxidase. The findings of this research work was similar with Jan et al. (1980) who also reported that specific activity increased after DEAE chromatography treatment as compared to \( \text{(NH}_4\text{)}_2\text{SO}_4 \) 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 Jan 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 \( \text{H}_2\text{O}_2 \) 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 to 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

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