

Activity of Guaiacol Peroxidase of *Solanum Lycopersicum* In presence of Detergents and Chaotropic Agents

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ABSTRACT: Browning of damaged tissues in fresh fruits and vegetables results mainly from the oxidation of phenolic compounds to quinones by peroxidase. The activation of Peroxidase by sodium dodesyl sulphat investigated, but reports on the effect of detergents and chaotropic agents on activity of Peroxidase are scarce. Here the effect of some detergents and chaotropic agent on activity of enzyme in soluble and in partial purified fraction was investigated. Sarkosyl inhibited activity of enzyme for oxidation of guaiacol and H₂O₂, although has inhibitory effect in higher concentrations. Other ionic, Nonionic detergents and Chaotropic agents showed inhibitory effect on activity of Peroxidase in partial purified fraction. Inhibitory effect of Urea, GnHCl, NP-40, Triton x-100 and Sodium cholate in presence of these substrates investigated so GnHCl and NP40 showed that they are potent inhibitor of peroxidase and sodium cholate showed that it is a weak inhibitor of guaiacol peroxidase. Results identified and confirmed the differences in structure and conformation of enzyme in soluble and in partial purified fraction for oxidation of different substrates.

KEYWORD: Catechol, Pyrogallol, Purification, Sarkosyl

I. INTRODUCTION

Peroxidase (EC 1.11.1.7; donor:hydrogen-peroxide oxidoreductase, POD) is one of the key enzymes controlling plant growth, differentiation and development. The enzyme participates in construction, rigidification and eventual lignification of cell walls, biosynthesis of ethylene from 1-aminocyclopropane-1-carboxylic acid and H₂O₂, regulation of auxin level through auxin catabolism, protection of tissue from damage and infection by pathogenic microorganisms, the oxidation of indoleacetic acid [1,2]. *In vitro*, this enzyme is widely employed in microanalysis [3]. More than 80% of immunoenzymatic kits contain peroxidase as labeling enzyme. Recently, peroxidases have been used for biotransformation of organic molecules [4]. It has been well established that peroxidase as one of the most stable enzymes can contribute to deteriorative changes in quality of the processed products [5]. Enzymatic browning is one of the most important colour reactions that affect fruits and vegetables. It is catalyzed by polyphenoloxidases and peroxidases [6]. It was estimated that over 50 percent losses in fruits and vegetables occur as result of enzymatic browning [7]. Marshall *et al.* 2000 [8] reported that the rate of enzymatic browning in fruits and vegetables is governed by the active polyphenoloxidase and peroxidase content of the tissues, the phenolic content of the tissues, pH, temperature and oxygen availability with the tissue. According to Khan and Robinson [9] the peroxidase is directly responsible for enzymatic browning in mangoes. The objective of this work was to study the effect of pH and heat on peroxidase activity and to extend the knowledge of vegetables peroxidase in particular.

II. MATERIALS AND METHODS

Chemicals: The small cherry tomatoes used in this study were obtained from Kurdistan of Iran and frozen at -25°C until used. Guaiacol, polyvinylpyrrolidone (PVPP), sodium cholate were purchased from Merck (Darmstadt, Germany). Phenylmethylsulfonyl fluoride (PMSF), nonaethylene glycol octylphenol ether (Triton x-100), nonaethylene glycol octylphenyl ether (NP-40), urea and guanidine chloride (GnHCl) were purchased from Sigma-Aldrich (St. Louis, USA). Sarkosyl was purchased from Fluka; All chemicals were of analytical grade.

Enzyme Extraction: 250 grams of wild pears were homogenized in 200 mL of 0.1M phosphate buffer (pH 6.8) containing 10 mM ascorbic acid and 0.5% polyvinylpyrrolidone with the aid of a magnetic stirrer for 1h. The crude extract samples were centrifuged at 30000 g for 20 min at 4°C. Solid ammonium sulphate (NH₄)₂SO₄ was added to the supernatant to obtain 30 and 80% (NH₄)₂SO₄ saturation, respectively. After 1 h, the precipitated proteins for each stage were separated by centrifugation at 30000 g for 30 min. The precipitate was redissolved in a small volume of distilled water and dialyzed at 4°C against distilled water for 24 h with 4 changes of the water during dialysis.

Protein Determination: Protein contents of the enzyme extracts were determined according to the Lowry method using bovine serum albumin as a standard [10].

Enzymatic activity assays: Partially purified Peroxidase activity was determined spectrophotometrically by following, at a specific wavelength, the increase in absorbance due to the oxidation of a selected substrate to its corresponding o-quinone. Namely, the increase in absorbance was followed at 420 nm in order to monitor the oxidation of guaiacol. Assays were conducted at room temperature (~ 22–25 °C), in a 3-ml reaction mixture prepared as follows: to 2.9–2.97 ml of 0.1 M phosphate buffer, pH 6.5, containing the appropriate amount of substrate prepared in the same buffer, an aliquot (75 µl) of Small cherry tomato extract was added. Enzymatic activity was determined by measuring the increase in absorbance at 420 nm for guaiacol with a spectrophotometer (6305 JENWAY). In order to correct for substrate autoxidation, the reaction mixture, was placed in the sample cuvette while the reference cuvette contained buffer and the substrate. Enzyme activity was calculated from the linear portion of the curve. Appropriate aliquots of 5 mM detergents and agents prepared in 0.1 M phosphate buffer, pH 6.8, were added to the reaction mixture just before addition of the small cherry tomato extract. The final volume of the reaction mixture was always 3 ml.

Effect of Detergents and Chaotropic Agents: The enzymatic activity was also measured in the presence of increasing concentrations of ionic detergents (Sodium cholate and sarkosyl), Nonionic detergents (Triton x-100 and NP-40) and Chaotropic agents (Urea and GmHCl). The concentrations used in each case were kept within the range where no aggregation was observed and were determined as a function of the sensitivity of the enzymatic activity. Enzymatic activity of Peroxidase assayed in extract and in partially purified Peroxidase separately.

Activity Assays in the presence of Ionic Detergents: The Peroxidase activity was assayed in 0.1 M phosphate buffer at pH 6.5 with 200 mM guaiacol and 10 mM of H₂O₂ as the substrates, respectively, in the presence of different concentrations of sarkosyl and sodium cholate. The concentration ranges remained below the critical micelle concentration (CMC) for each detergent.

Activity Assays in the presence of Non Ionic Detergents: The Peroxidase activity was assayed in 0.1 M phosphate buffer at pH 6.5 with 200 mM guaiacol and 10 mM of H₂O₂, respectively, as the substrates, in the presence of different concentrations of Triton x-100 and NP-40. The concentration ranges remained below the critical micelle concentration (CMC) for each detergent.

Activity Assays in the presence of chaotropic Agents: The Peroxidase activity was assayed in 0.01 M phosphate buffer at pH 6.5 with 200 mM guaiacol and 10 mM of H₂O₂ as the substrates in the presence of different concentrations of urea or GmHCl. PEROXIDASE activity assayed at extract and partially purified Peroxidase, separately.

III. RESULTS

Assays conducted with small cherry tomatoes crude extract at pH 6.5: Peroxidase activity in presence of guaiacol and H₂O₂ assayed as described in materials and methods, were detectable in tomatoes extract. Under our experimental conditions, no lag period was observed in the expression of either activity.

Effect of Ionic detergents. Effect of sarkosyl : The addition up to 0.3 mM sarkosyl to the reaction mixture resulted up to a 25% decrease in the activity when guaiacol and H₂O₂ were used as the substrate at pH 6.5 (Figure 1). Higher detergent concentration up to 1 mM caused a 45% decrease in Peroxidase activity with guaiacol and H₂O₂ as the substrates. Over the concentration range (0-1 mM), sarkosyl had an inhibitory effect on Peroxidase activity in presence of guaiacol and H₂O₂, although higher concentrations of sarkosyl, cloudiness prevented further investigations with these substrates (table 1).

Effect of sodium cholate: In the presence of sodium cholate at pH 6.5, a decrease in Peroxidase activity was observed when guaiacol and H₂O₂ were used as the substrate. The inhibition increased with increasing detergent concentration, until a plateau was reached. In the presence of sodium cholate (1 mM), the Peroxidase activity decreased progressively to 80% of the control. The detergent concentration increased progressively to 1 mM in presence of guaiacol and H₂O₂. Peroxidase activity reached to 65%, so, showed 20% inhibition of Peroxidase. In the presence of higher sodium cholate concentrations (3 mM), the Peroxidase activity was reduced to 65% of the control (Figure 2).

Effect of Nonionic detergents at pH 6.5: The addition of Triton x-100 or NP-40 to the reaction mixture at pH 6.5 led to a decrease in the Peroxidase activity, whether guaiacol and H₂O₂ were used as the substrates. The extent of inhibition, however varied with the detergent used. With guaiacol and H₂O₂, up to 75% inhibition was observed in the presence of 2 μM of triton x-100. 70% inhibition was observed in the presence of 2 μM NP-40 (Figure 3). Over the same ranges of concentrations (4 μM for triton x-100 and NP-40), no effect was observed on Peroxidase activity when guaiacol and H₂O₂ were used as the substrate. Activity of Peroxidase decreased in presence of triton-100 and NP-40 as detergents.

Effect of Chaotropic Agents: The addition of increasing of urea to the assay mixture at pH 6.5 resulted in increasing inhibition of the Peroxidase activity, so that 50% inhibition was reached with 100 mM urea and increasing of urea concentration up to 300 mM caused only 70% inhibition in Peroxidase activity in presence of guaiacol and H₂O₂; no further inhibition was observed for up to 350 mM urea (Figure 4).

Effect of GmHcl: Up to 45% and 70% inhibition of Peroxidase activity was observed for catechol oxidation in presence of 100 and 300 mM GmHcl, respectively. No further inhibition was observed for up to 350 mM urea (Figure 4).

Table 1 : Effect of Detergents and Chaotropic agents on Peroxidase activity of solanum lycopersicum in extract at pH 6.5

<i>Detergents</i>		<i>Concentrations</i>	<i>Effect on Catechol oxidation</i>
<i>Ionic detergent</i>	<i>Sarcosyl</i>	<i>0-0.3</i>	<i>25 % Inhibition</i>
		<i>0-0.9</i>	<i>55 % Inhibition</i>
	<i>Sodium cholate</i>	<i>0-1</i>	<i>22 % Inhibition</i>
		<i>0-2.5</i>	<i>34 % Inhibition</i>
<i>Non ionic detergent</i>	<i>Tx-100</i>	<i>0-1 μM</i>	<i>42 % Inhibition</i>
		<i>0-4 μM</i>	<i>75 % Inhibition</i>
	<i>NP-40</i>	<i>0-1 μM</i>	<i>36 % Inhibition</i>
		<i>0-4 μM</i>	<i>72 % Inhibition</i>
<i>Chaotropic detergent</i>	<i>Urea</i>	<i>0-10 mM</i>	<i>15 % Inhibition</i>
		<i>0-250 mM</i>	<i>68 % Inhibition</i>
	<i>GmHcl</i>	<i>0-10 mM</i>	<i>19 % Inhibition</i>
		<i>0-250 mM</i>	<i>73% Inhibition</i>

IV. DISCUSSION

The present investigation was conducted on extracts obtained from small cherry tomato. The extract prepared as described in the Materials and Methods consisted of the soluble fraction of the fruit homogenate and the Peroxidase activity reported here refer to the soluble enzyme. Each assay was performed at least in triplicate and each experiment was repeated three times. The results were expressed as means of the values obtained and the standard deviations of the means were calculated and shown on the figures. Peroxidase retains its intriguing property of being able to exist in a latent form which it can be released by a variety of treatments, such as exposure to activators that are reputed to inactive enzymes [11]. Sarkosyl would inhibit small cherry tomato Peroxidase activity for oxidation guaiacol and H₂O₂ exhibited different sensitivities toward the various detergents and agents used. Peroxidase activity for oxidation of guaiacol and H₂O₂ was sensitive to detergent/agent concentrations the same as activity of Peroxidase for pyrogallol and catechol oxidation [12]. Our results showed that among the detergents/agents tested in this study sarkosyl would inhibit Peroxidase the same as other detergents that inhibited the enzyme activity. The inhibition observed with sodium cholate may be due to steric hindrance upon binding of this bulkier detergent to the enzyme. Nonionic detergents were larger than sodium cholate and may cause inhibition upon binding to the enzyme by preventing access of the substrate to the active site because of steric hindrance [19]. The present study shows that Peroxidase in *solanum lycopersicum* are inhibited by sarkosyl and by urea and other detergents. Interestingly, while no more than 70% inhibition was observed in the presence of up to 300 mM urea for oxidation of guaiacol and H₂O₂. Similarly, GmHcl caused no more than 75% inhibition of Peroxidase for oxidation of guaiacol and H₂O₂. Thus activity of soluble Peroxidase of *solanum lycopersicum* was sensitive to chaotropic agents and nonionic detergents for oxidation of these substrates. These findings indicated probably a difference in the conformational and in the microenvironment of the respective active site in the enzyme.

V. ACKNOWLEDGMENT

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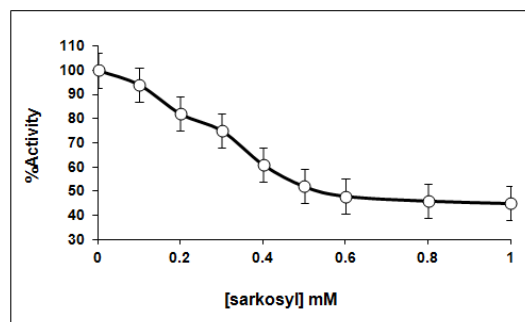


Figure 1. Effect of sarkosyl concentrations on small cherry tomato peroxidase activity in presence of guaiacol 200 mM and H₂O₂ 10 mM. The reaction medium at 25 °C contained 0.1M sodium phosphate buffer, pH 6.5 and increasing concentrations of sarkosyl(0 to 1 mM).

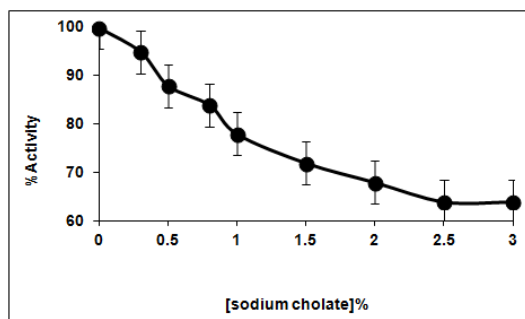


Figure 2. Effect of sodium cholate concentrations on small cherry tomato peroxidase activity in presence of guaiacol 200 mM and H₂O₂ 10 mM. The reaction medium at 25 °C contained 0.1M sodium phosphate buffer, pH 6.5 and increasing concentrations of sodium cholate(0 to 3 mM).

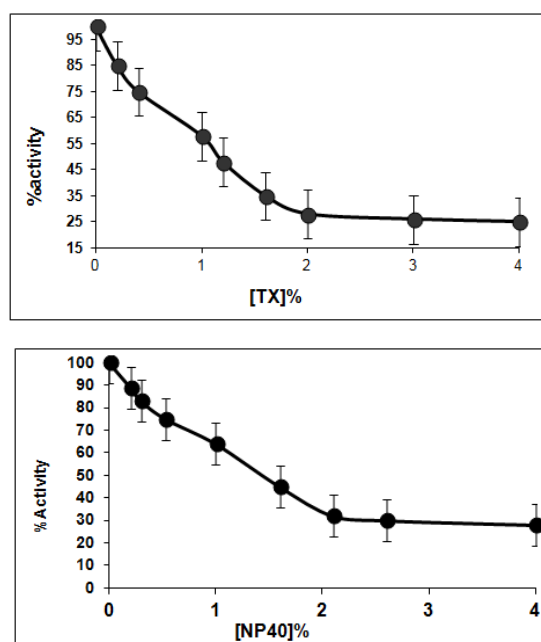


Figure 6. Effect of NP-40 and Triton x-100 concentrations on partial purified peroxidase activity of small cherry tomato in presence of pyrogallol 15 mM. The reaction medium at 25 °C contained 0.1M sodium phosphate buffer, pH 6.8 and increasing concentrations of Triton x-100 and NP-40(0 to 5 μ M).

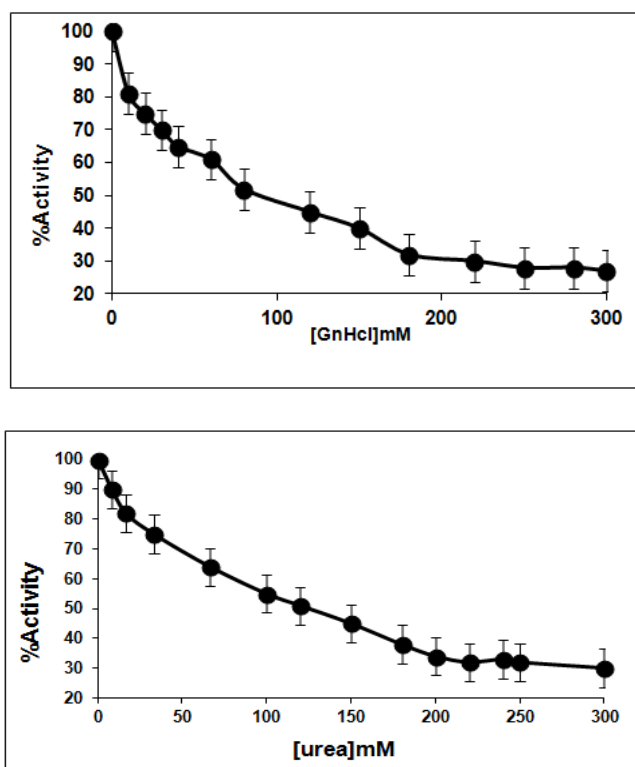


Figure 4. Effect of guanidine hydro chloride (Gn.Hcl) and Urea concentrations on small cherry tomato peroxidase activity in presence of pyrogallol 15 mM. The reaction medium at 25 °C contained 0.1M sodium phosphate buffer, pH 6.8 and increasing concentrations of Gn.Hcl and urea(0 to 350 mM).