INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2000/02–4–348–350

Activity and Kinetics of Peroxidase in Ripe and Unripe Tomatoes

M.A. MALANA, R. ZOHRA, M. YAQUB† AND KHALIL-UR-REHMAN†
Department of Chemistry, Baha-ud-Din Zakariya University, Multan, Pakistan
†University of Agriculture, Faisalabad–38040, Pakistan

ABSTRACT

Crude peroxidase (POD) extracts from ripe and unripe tomatoes were partially purified by $(NH_4)_2SO_4$ precipitation method. The purification factor found to be 15.8 in unripe form and 0.6308 in ripe form .The POD activity was greater in unripe tomatoes (18.34μ/ml) than that in ripe tomatoes (1.31 μ/ml). The enzyme followed Michaealis-Menton mechanism mode. Calculated values for V_{max} and K_m were 2.86 absorbance units/min and 0.971 mM, respectively.

Key Words: Tomatoes; POD; Partial purification; Enzyme kinetics

INTRODUCTION

In biochemical, biomedical and cytological researches, peroxidase (POD) is being used as a macro-molecular tracer whose path can be followed histochemically in the organism. POD being the most heat stable enzyme in plants, is very resistant to thermal inactivation, and hence widely used as an index of blanching procedures in food industry (Reed, 1975). Nevertheless, no remarkable work on this enzyme has so for been done in Pakistan. This paper describes the POD activity in tomatoes.

MATERIALS AND METHODS

The comparative POD activity in the crude and partially purified extracts of ripe and unripe tomatoes was studied. The procedure adopted by Talat (1996) and Theorell (1942) was followed. Pure standard horseradish POD (SIGMA, USA) of RZ value 3.04 was used as standard. The POD activity of the unripe and ripe tomatoes was measured by using guaiacol (1.11 gm/ml density) as chromogen on spectrophotometer. The extract free of all cellular components was heated at 65°C for three minutes in a water bath and then cooled promptly by placing in ice bucket for inactivation of catalase activity.

Partial purification of POD from ripe and unripe tomatoes. Partial purification of POD was done by using $(NH_4)_2SO_4$ (Evan, 1968; Ginello *et al.,* 1995). The experiments for enzyme extraction were carried out at room temperature and distilled water was used as solvent. Crude enzyme was 50% saturated by adding 70 g of solid $(NH_4)_2SO_4$ to 200 ml of the crude extract. The solution was centrifuged at 10,000 rpm for 15 minutes. The supernatant was adjusted to 85% $(NH_4)_2SO_4$ saturation by adding more appropriate amount of solid $(NH_4)_2SO_4$. The solution was again centrifuged at 10,000 rpm for 15 minutes and filtered through Wattman 1 filter paper. Supernatant was discarded and precipitates were dissolved in 20 ml of phosphate buffer of pH 6.

Protein was estimated by using biuret reagent (Sheikh, 1991); max for standard POD was determined by plotting the absorbance values at different wavelengths. Five ml of the buffered substrate solution was taken in 14 test tubes. Absorbance was noted at 420 nm with 3 min. Reaction period after adding standard POD (1:40) in each test tube one by one was noted.

Michealis-Menton equation was confirmed by using partially purified enzyme, POD from unripe tomato and guaiacol as substrate. 0.1 ml of extract was added in different volumes of substrate solution with increasing concentration of 0.913, 1.096, 1.279, 1.461, 1.644, 1.827, 2.009 mM and absorbance was noted. A double reciprocal plot was obtained by plotting 1/V against 1/[S]. V_{max} and K_m were calculated from the graph.

RESULTS AND DISCUSSION

The concentration of protein in unripe tomatoes was less (0.218 mg/ml) than that of ripe tomatoes (0.30 mg/ml). On the other hand, partially purified extract of unripe tomatoes had more protein concentration (0.227 mg/ml) than that of ripe tomatoes (0.155 mg/ml) indicating that unripe tomatoes are good source of said enzyme. Different dilutions of standard POD, i.e. 1:20, 1:40, 1:60, 1:80, 1:100, 1:120, 1:140, 1:160, 1:180 and 1:200, showed the absorbance values 0.204, 0.224, 0.244, 0.284, 0.317, 0.445, 0.564, 0.704, 1.064 and 2.091, respectively. Standard curve was calibrated by plotting a graph between absorbance values and

concentrations of POD i.e. 5.5, 6.0, 6.9, 7.7, 8.8, 11.0, 13.8, 18.4, 27.5, and 55.0 units/ml in respective dilutions (Fig. 1). POD activity in crude extract (1:10) of unripe tomatoes was 1.102 units/ml. The concentrations of POD for partially purified extracts of unripe and ripe tomatoes were 18.34 units/ml and 1.31 units/ml, respectively; whereas, the POD concentration in crude extract of ripe tomatoes was 4.023 units/ml.

Fig. 1. Standard curve for peroxidase activity

Partial purification of POD. The purification factor was found to be 15.8 in the unripe tomatoes and 0.6308 in the ripe tomatoes (Table I). This difference may be explained on the fact that the amount of POD activity in plants varies in relation with the anatomical locations (Evan & Aldridge, 1965; Rahayungish, 1990), physiological activity (Joslyn & Bedford, 1940), age of tissue or plant (Nam *et al.,* 1991) and state of being fresh or stocked.

Table I. Purification factor of peroxidase from tomatoes

EE	PC mg/mL	EA u/mL	TP	TA	SA mg/mL	PF
Unripe tomatoes						
Crude	0.218	1.102	43.6	220.4	5.1	1
Purified	0.217	18.34	4.54	366.8	80.7	15.8
Ripe tomatoes						
Crude	0.300	4.023	6.0	804.6	13.41	1
Purified	0.155	1.312	3.1	26.24	08.46	0.6308

EE= Enzyme extracts; PC= Protein concentration; EA= Enzyme activity; TP= Total protein; TA= Total activity; SA= Specific activity; PF= Purification factor

The value of max (470 nm) at different wavelengths is given in Fig. 2. Enzyme activity at varying concentrations of POD have been presented in Fig. 3. Initially the increase in enzyme activity with increase of enzyme concentration was greater and gradually the difference was in decreasing order, because of limited availability of substrate.

Effect of substrate concentration on POD activity (Michealis menton equation). Effect of substrate concentration on POD activity at substrate concentrations of 0.913, 1.096, 1.279, 1.461, 1.644, 1.827 and 2.009 mM has been depicted in Fig. 4. Calculated value for V_{max} was 2.86 absorbance units/min and average value for K_m was 0.971 mM. Straight line of the Line-Weaver Burk plot confirms the obedience of POD for Michealis-Menton equation (Fig. 5).

Fig. 2. Maximum wavelength for peroxidase activity

Fig. 3. Absorbance values for different enzyme concentrations in unriped tomatoes

Fig. 4. Substrate concentration on peroxidase activity

Fig. 5. Line-Weaver Burk Plot

REFERENCES

Evan, J.J., 1968. PODs from the extreme dwarf tomato plant: Identification, isolation and partial purification. *J. Plant Physiol.,* 43: 1037–41.

Evan, J.J. and N.A. Aldridge, 1965. The distribution of PODs in extreme dwarf and normal tomato. *J. Phytochem.,* 43: 499–503.

Joslyn, M.A. and C.L. Bedford, 1940. Enzyme activity in frozen vegetable, Aspergus. *Indian Chem.,* 32: 702–4.

Nam, S.H., S.U. Choi and M.S. Yang, 1991. Changes and characteristics of the biochemical components of soybean cell tissue culture. *Hanguk Nonghwahak Hoechi,* 34: 134–41.

Rahayungish, S.A., 1990. POD activities and their relationship to resistance of rapper plants to phytophthora palmivora. *Indus. Crops Res. J.,* 3: 18–22.

Reed, G., 1975. *Oxidoreductases: Enzymes in Food Processing*. Academic Press, New York, p. 216.

Sheikh, M.A., 1991. A study of non-enzymic glycosylation. *Ph.D. Thesis,* Strathclyde University, Glassgow, U.K.

Talat, T., 1996. Studies on comparative evaluation of POD extracted from turnip and radish. *M.Sc. Biochemistry Thesis,* Deptt. of Chemistry, Univ. Agri. Faisalabad, Pakistan.

Theorell, H., 1940. *Chemistry and Methods of Enzymes.* 2nd Ed. Academic Press. New York, p. 207

Theorell, H., 1942. Plant peroxidase. *Enzymologia,* 10: 250.

(Received 04 April 2000; Accepted 08 September 2000)