How Metals and Amino Acids Effect Guaiacol Peroxidase Activity from Dill

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Abstract—*Environmental pollution has been a serious problem since the start of the industrial revolution. The pollution usually comes from industrial wastes, waste water, heavy metal, etc. Heavy metals such as lead, mercury, tin are considered to be toxic to living organisms and enzyme activities. However, metal ions can play essential role in plants and animals as they are involved in fundamental processes such as photosynthesis, respiration and DNA synthesis. Plants with their antioxidant enzymes which are in plant defense system show some stress when they are exposed the metals. One of the iron-heme dependent antioxidant enzymes is peroxidase (POD) enzyme that catalyzes hydrogen peroxide mediated oxidation of different substrates such as phenols. In the present work, Guaiacol peroxidase (POD) was first characterized from the dill (Anethum Graveolens L.) with the optimum pH and temperature. Then, the effects of various metals and amino acids on the dill Guaiacol peroxidase (POD) activity were investigated. The POD activity was tested against eight different metals (Hg(II), Co (II), Cur(II), Fe(III), Mn(II), Pb(II), Zn(II) and Sn(II)) and five different amino acids (L-Cysteine, L- Phenylalanine, L-Gysine, L- Arginine and L-Aspartic acid) with hydrogen peroxide and guaiacol. According to the results, Cu(II), Mn(II) and Fe(III) increase the POD activity, Co(II), Zn(II) and Pb(II) have almost no effect the enzyme activities and Hg(II) and Sn(II) decrease the POD activity. The tested amino acids had inhibitory effect to dill Guaiacol POD activity.*

Keywords— Amino acids, metals, peroxidase, dill.

I. INTRODUCTION

Soil pollution is a serious problem for environmental. It comes from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded paints, fertilizers, sewage sludge, pesticides, wastewater irrigation, etc. [1]. The pollution can damage plants, animals, and human health as well as microorganisms and insects living in the soil. Toxic heavy metals are commonly found at contaminated sites are lead (Pb), chromium (Cr), arsenic (As), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni) [2]. These have metal pollutants found in the soil can cause their deleterious. The heavy metals can lead to oxyradical production which then causes oxidative stress in plants and soil organisms. They can bind antioxidant enzymes (Superoxide peroxidase, Catalase, GSH reductase/ peroxidases) and reduce the antioxidant ability of plants and organisms. They can also compete for metal-cofactor binding of metallo-enzymes and cause their inactivation [3]. However, there are also such metals as cooper, iron, zinc, calcium and magnesium which are essential for plant nutrient that they are involved in fundamental surviving processes such as photosynthesis, DNA synthesis and hormone formation [4] Plants with their antioxidant enzymes which are involved in plant defense system show some stress when they are exposed the metals. One of the iron-heme dependent antioxidant enzymes is peroxidase (POD) enzyme. Peroxidase (POD) (oxidoreductase, EC: 1.11.1.7) catalyze the oxidation of a wide variety of phenolic compounds such as guaiacol, pyrogallol, acid chlorogenic, catechin and catechol in the presence of hydrogen peroxide or organic hydroperoxides [5-6].

In the present work, Guaiacol peroxidase was first isolated and characterized from dill (*Anethum Graveolens L.*). Then, the effects of various metals and amino acids on the dill Guaiacol peroxidase (POD) enzyme activity were investigated.

II. MATERIALS AND METHODS

2.1 Chemicals

Dill (*Anethum Graveolens L.*) used in this study was obtained from Sakarya region and stored at –20°C until used. Polyviniylpolypyrolidone (PVP), Sephadex G-100, $(NH_4)_2SO_4$ and other chemicals were obtained from Sigma Chemical Co., St. Louis, MO.

2.2 Extraction and purification

30 g of the dill *(Anethum Graveolens L.)* was obtained from local Sakarya region. After that samples were added to 10 ml 50mM sodium phosphate buffer (pH; 7.0), 0.3 g polyvinylpolypyrolidone (PVPP), and extraction was prepared. The mixture was homogenized with blender. After the filtrate was centrifuged at 14.000xg for 30 min and supernatant was collected. Extraction was

fractionated with $(NH_4)_2SO_4$, solid $(NH_4)_2SO_4$ was added to the supernatant to obtain 80% saturation. The mixture was centrifuged at 14,000xg for 30 minutes and the precipitate was dissolved in a small amount of phosphate buffer and then dialyzed at 4°C in the same buffer for 24 h with three changes of the buffer during dialysis. The dialyzed enzyme extract was centrifuged and loaded onto Sephadex G-100 column previously equilibrated with extraction buffer, and washed with the same buffer to remove unbound proteins. The eluate was used as the POD enzyme source in the following experiments. The amount of POD was performed according to method of Bradford with bovine serum albumin as standard [8].

2.3 Enzyme assay

Guaiacol POD activity assay was performed as in Koksals method [7] with slight modifications. Guaiacol was used as a phenolic substrate by measurement of the absorbance at 470 nm of 3,3´-Dimethoxy-4,4´-biphenoquinone (ε = 6.39/mM/cm) at room temperature. The reaction mixture was prepared with sodium phosphate buffer, pH 6.5 (0.3 M), guaiacol (45 mM), H_2O_2 (22.5 mM) and 25 µl of enzyme solution. One unit of enzyme activity was defined as the amount of enzyme catalyzing the production of 1 mmol of 3,3´-Dimethoxy-4,4´-biphenoquinone per min.[9]. For each substrate (guaiacol and H_2O_2), the kinetic data were plotted as reciprocals of activities versus substrate concentrations. The Michaelis-Menten constant (Km) and maximum velocity (Vmax) were determined as the reciprocal absolute values of the intercepts on the x- and y-axes, respectively, of the linear regression curve. Substrate specificity (Vmax, Km) was calculated by using the data obtained on a Lineweaver-Burk plot [10].

2.4 Effect of pH and Temperature

POD activity, as a function of pH, was determined in a pH range of 4.5–5.5 in 50 mM acetate buffer, 6.5–7.5 in 50 mM phosphate buffer and 8.5–9.5 in 50 mM Tris–HCl and Tris-Base buffer. The optimum pH values obtained from this assay were used in all the other experiments. The effect of temperature on POD activity obtained at different temperature values (10-80ºC) and the optimum temperature of poopy POD was determined.

2.5 Effect of Metals and Amino acids

Eight different metals Hg(II) (Mercury(II)Sulphate), Co(II) (Cobalt(II)Chloride), Cu(II) (Cooper(II)Sulphate), Fe(II) (Iron(III)Chloride), Mg(II) (Manganese(II)Sulphate), Pb(II) (Lead(II)Chloride), Zn(II) (Zinc(II)Sulphate), Sn(II) ,(Tin(II)Chloride) at 5 mM concentrations were used to determine their effects on Dill POD enzyme activity with hydrogen peroxide and guaiacol as substrats

The effect of five different amino acids (L-Cysteine, L-Tyrosine, L-Gysine, L- Arginine and L-Aspartic acid with different concentrations (1-50 mM)) on dill POD activity was determined by using hydrogen peroxide and guaiacol as substrates at 470 nm in UV-vis spectrophotometer. The remaining enzyme activities were calculated for all the metals and amino acids.

III. RESULTS AND DISCUSSION

Guaiacol Peroxidase from the dill (*Anethum Graveolens L.*) was isolated and kinetically characterized by using guaiacol and H_2O_2. The effect of pH on the enzyme was determined by measuring the activity at different pH range of 3.0–9.0 [Fig. 1]. The enzyme showed the highest activity in pH 6.5. A similar optimum pH for the POD has been reported from turnip, Horseradish legumes, Horseradish roots [11].

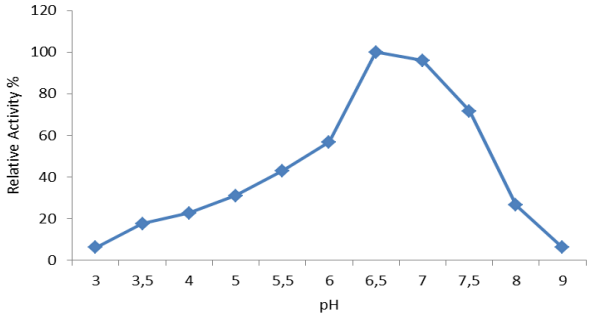

Fig 1.0: Effect of pH on Dill Guaiacol-POD activity

The effect of temperature on the guaiacol POD was studied at different temperatures from 10°C to 80°C. The optimum temperature of the POD was found to be 40°C for guaiacol [Fig. 2]. A wide variability in the optimum temperatures has been reported for POD from various sources. The optimum temperatures have been changed from 30 to 60°C [12, 13].

Km and Vmax values were calculated for guaiacol and H_2O_2 substrates from Lineweaver-Burk graphs. The Km values of the POD for guaiacol and H_2O_2 substrates were 18.6 and 2.3 mM, respectively. Indeed, Vmax values were 0.202 and 0.311 mM/min for above mentioned substrates, respectively [Table 1].

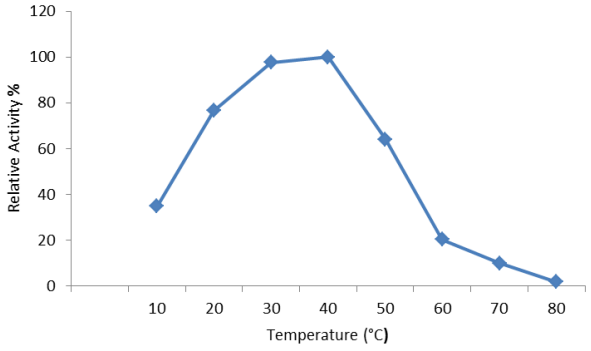

Fig. 2.0: Effect of temperature on Dill Guaiacol-POD activity

Table I: Optimum activity conditions of dill Guaiacol POD

Substrates	Km (mM)	Vmax (mM/min)	Optimum pH	Optimum Temperature (°C)
Guaiacol	18.6	0.202	6.5	40
H_2O_2	2.3	0.311	6.5	40

The effect of eight different metals on dill guaiacol POD enzyme activity are determined. The results are shown in Fig. 3. Fe (II), Cu(II) and Mn(II) are slightly increased the POD activity. POD enzyme is a metallo enzyme and includes hem group with iron. Some POD enzymes have Mn in its active site [14]. These metals can effect and increase the enzyme activity with interacting the active site of POD enzyme which is a metallo enzyme. Co(II), Zn(II) and Pb(II) have almost no effect the enzyme activity and Hg (II) and Sn(II) decrease the POD activity. These metals are known as heavy and toxic metals because they inhibit the enzyme activity with high efficiency. It is believed the action of metals is due to its interaction with the iron at the active center of the enzyme.

The effects of five different amino acids were also tested against dill guaiacol POD activity. The tested amino acids showed inhibitory effect to dill POD activity. The I_{50} values of these amino acids were found to be 15.2, 5.9, 21.5, 18.3 and 5.2 mM for glycine, arginine, aspartic acid, phenylalanin and cystein respectively (Table 1). L-cysteine (I_{50} value 5.20 mM) was the most effective inhibitors.

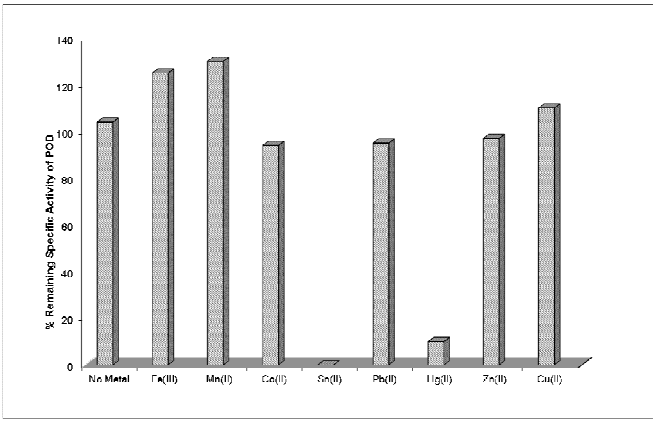

Fig. 3.0: % Remaining specific activity profiles for POD enzyme from Dill (Anethum Graveolens L.) against metals

Table 2.0: Effect of L-amino acids on Dill POD enzyme activity

Aminoacids	I (mM)	I (%)	I_{50} (mm)
L- Glysine	5	30	
	10	43	15.2
	20	62	
L-Arginine	1	22	
	5	43	5.9
	10	65	
L-Aspartic Acid	5	16	
	10	48	21.5
	20	65	
L-Phenylalanine	10	29	
	25	48	18.3
	50	59	
L-Cysteine	1	28	
	5	45	5.2
	10	65	

IV. CONCLUSION

In this work, guaiacol peroxidase (POD) enzyme first was isolated and characterized from dill (*Anethum Graveolens L.*). Then the effects of heavy metals showed that Cu(II), Mn(II) and Fe(III) increase the POD activity. These metals can effect and increase the enzyme activity with supporting and interacting the metal in active site of POD enzyme , Zn(II) and Pb(II) have almost no effect the enzyme activities and Hg(II) and Sn(II) decrease the POD activity. These heavy metals are known to be toxic for living organism and plants. POD is an enzyme involving defense system of plants and animals. The decreasing the POD enzyme activity could cause less defense system of dill plant against heavy metal environment. The results obtained in the present study could be useful for understanding how metals can affect the antioxidative defense system of dill plant. The tested amino acids had inhibitory effect to dill POD activity. L-cysteine was the most effective inhibitor for dill guaiacol POD. Due to its nontoxic properties, cysteine may be promising

alternatives to sulfite in inhibition of POD enzyme of plant foods. Thus, our study introduces a new source of guaiacol POD and its behaviors to metals and amino acids that can be used in the environment, medicine, chemical and food industries.

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