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NOTE

Inhibition of Potato Polyphenol Oxidase by Halides

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The inhibition of potato polyphenol oxidase catalyzed oxidation of catechol by potassium halides has been carried out on the basis of kinetic measurement. The concentration of catechol was varied in the range of 1.71–8.60 × 10^{-3} M and that of inhibitor was maintained 4.38 × 10^{-4} M. The apperent Michaelis constant, K_{mapp} and dissociation constant of enzyme-inhibitor complex, k_i were determined at pH 6.80 and temperature 27.0°C. The potassium halides show competitive type of inhibition. Further, the effect of pH on inhibition was also investigated.

Key Words: Potato Polyphenol oxidase, Catechol, Potassium halide inhibitors.

The enzyme polyphenol oxidase of potato is often responsible for the gradual darkening of potato whenever it is cut open and exposed to air. It would be desirable if some ways and means are known to suppress and inhibit this darkening.

The inhibition of mushroom polyphenol oxidase by ascorbic acid has been studied by few workers[1–3] and reported inactivation of enzyme while Krueger[4] showed the activation of enzyme by ascorbic acid. Duckworth and Coleman[5] showed that cynide and benzoic acids are the competitive inhibitors of the polyphenol oxidase. Similar finding has also been reported by Lerner[6]. Effdinger *et al.*[7] showed that nitrocatechol is a competitive inhibitor of the enzyme.

Since the inhibition of potato polyphenol oxidase by potassium halides has not been studied, it is proposed to study the inhibition of potato polyphenol oxidase by potassium chloride, potassium bromide and potassium iodide.

All the glasswares used in the study were cleaned with teapol, washed repeatedly with hot distilled water and rinsed with double distilled water before being used for preparing the solutions. The chemicals used were of analytical grade.

The enzyme potato polyphenol oxidase was extracted by the known procedure[8]. Phosphate buffer was used to maintain the pH of the system. Catechol, potassium halides, oxygen and enzyme solution were prepared in the buffer solution. The concentrations of each in the reaction system were 8.60 × 10^{-3}, 4.38 × 10^{-4}, 2.86 × 10^{-3} and 5.0 × 10^{-6} M, respectively.

In a typical kinetic experiment, 10 mL catechol (0.030 M) was mixed with 20 mL oxygen saturated buffer solution and the mixture was kept in stoppered bottle. Another stoppered bottle containing 5 mL of potato polyphenol oxidase solution was taken and both the bottles were kept in a thermostat at 27.0°C. After 30 min, the two solutions were mixed and quickly transferred into the cuvette. The absorbance was measured at λ_{max} nm at various intervals of time.

From the observed absorbances at various intervals of time the corresponding concentration of *o*-quinone could be evaluated. From these results, the initial rate of reaction was evaluated. Similar studies were carried out at various catechol concentrations, keeping all other parameters same.

It was observed that potassium chloride, potassium bromide and potassium iodide did have an inhibiting effect on oxidation of catechol catalyzed by potato polyphenol oxidase. In the presence of these salts there was considerable decrease in the initial rate of oxidation of catechol (Table-1). From the observations it is clear that potassium iodide has the least effect and potassium chloride has the strongest effect.

TABLE-1
INITIAL RATE OF POTATO POLYPHENOL OXIDASE CATALYZED OXIDATION OF CATECHOL BY OXYGEN; INHIBITION BY POTASSIUM HALIDES AT VARIOUS CATECHOL CONCENTRATIONS

[inhibitor]	: 4.38×10^{-4} M
[potato polyphenol oxidase]	: 5.00×10^{-6} M
[oxygen]	: 2.86×10^{-3} M
Temperature	: 27.0°C
pH	: 6.80

Inhibitor	Initial rate, V (10^{-5} Ms^{-1})				
	Catechol concentration (10^{-3} M)				
	8.60	6.86	5.10	3.43	1.71
—	2.82	2.76	2.69	2.56	2.21
Potassium chloride	2.29	2.17	1.96	1.66	1.15
Potassium bromide	2.49	2.39	2.24	1.98	1.47
Potassium iodide	2.53	2.48	2.32	2.08	1.58

K_m and V_{max} values were evaluated from the lineweaver-Burk plot. In all the cases it was observed that while V_{max} remained unaltered, the K_m changes over to K_{mapp}. Evidently these were the cases of competitive inhibition. From the values of K_m and K_{mapp} the dissociation constant of enzyme inhibitor complexes, k_i were also evaluated (Table-2). As all are the cases of competitive inhibition, these inhibitors compete with the substrate for the active site of the enzyme.

From the observed values of dissociation constants of the enzyme inhibitor complexes, k_i, it could be inferred that the binding of potassium chloride to active site was strongest while that of potassium iodide was the weakest.

From the results of effect of pH (Table-3), it was observed that the percentage inhibition in the basic media was more as compared to acidic media. At high pH the quilibrium for oxidation of catechol shifted to right and hence catechol is highly susceptible to oxidation. Therefore, in presence of inhibitors the oxidation is

seriously affected so that the % inhibition is high. In contract, at low pH the tendency of oxidation itself is very much less so that the % inhibition also is distinctly less. At each of the three pH values the observed trend of increase in % inhibition was potassium chloride > potassium bromide > potassium iodide.

TABLE-2
K_m, K_{mapp} AND k_i FOR POTATO POLYPHENOL OXIDASE CATALYZED OXIDATION OF CATECHOL BY OXYGEN IN ABSENCE AND PRESENCE OF INHIBITORS

[inhibitor]	: 4.38×10^{-4} M
[potato polyphenol oxidase]	: 5.00×10^{-6} M
[oxygen]	: 2.86×10^{-3} M
Temperature	: 27.0°C
pH	: 6.80

Inhibitor	K_{mapp} (10^{-3} M)	Dissociation constant of enzyme-inhibitor complex, k_i (10^{-4} M)
—	0.60	—
Potassium chloride	3.03	1.08
Potassium bromide	1.76	2.27
Potassium iodide	1.69	2.41

TABLE-3
EFFECT OF pH ON INHIBITION

[inhibitor]	: 4.38×10^{-3} M
[potato polyphenol oxidase]	: 5.00×10^{-6} M
[oxygen]	: 2.86×10^{-3} M
[catechol]	: 8.60×10^{-3} M
Temperature	: 27.0°C

Inhibitor	% Inhibition			Increase in % inhibition
	pH			
	6.40	6.80	7.20	
Potassium chloride	10.8	18.8	26.5	8.0
Potassium bromide	5.4	11.7	17.1	6.3
Potassium iodide	4.6	10.3	15.5	5.7

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