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INHIBITION OF PALMITO POLYPHENOLOXIDASE BY HALIDE SALTS

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The inhibitory properties of halide salts on palmito polyphenoloxidase (PPO) are described. Halide salts have the same inhibitory effect on the two forms of palmito PPO separated by hydrophobic chromatography. Fluoride and chloride ions showed a non-competitive, mixed type inhibition while bromide and iodide ions were found to be non-competitive inhibitors. A study of the K_i for the different halide salts showed that the smaller F^- ion is a stronger inhibitor than I^- and Br^- and that Cl^- has the highest K_i value. This suggests that the active site of the palmito PPO is not easily accessible. The inhibition by chloride and fluoride ion was found to be pH-dependent. The inhibitory effects of these ions increased with a decrease in pH. It is suggested that halide ions (X) could bind to either the protonated enzyme (EH) or the protonated substrate–enzyme complex (EHS) to yield inactive forms EHX and EHSX, respectively.

Keywords: Polyphenoloxidase; *Acanthophoenix rubra*; Halide inhibition; pH study

INTRODUCTION

Unfavourable enzymatic browning of many food products has been of great concern to food technologists and processors. Polyphenoloxidase (EC 1.14.14.1; PPO) is the main enzyme that causes food browning and

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has been the subject of several reviews.[1-4] Many reports have shown the inhibitory effect of halide ions on different polyphenoloxidase systems.[5-8] This inhibitory effect was reported to be pH-dependent where inhibition increased with a decrease in pH, with maximum inhibition occurring in the range pH 3.5–5. It has been suggested that pH modified interactions between the positive charge on an imidazole functional group located in the active site and the negatively charged halide ion.

Two forms (H1 and H2) of palmito PPO were purified by hydrophobic chromatography.[9] These forms have different molecular weights (67,450 and 49,900 respectively for H1 and H2) and have some kinetic parameters that differ. The present study was therefore undertaken to investigate the inhibitory effect of halide salts on palmito PPO, the types of inhibition concerned and to study the influence of pH on inhibition by fluoride and chloride ions.

MATERIAL AND METHODS

Substrates and reagents were supplied by Sigma (St Louis, MO). Palmito PPO was purified according to Robert *et al.*[9]

Assay

For routine analysis, the substrate was 4-methylcatechol (20 mM) in 3 ml of a McIlvaine's buffer solution at pH 5. PPO activity was polarographically assayed with a Clark electrode using air-saturated substrate solution at 30°C. The rate of the reaction was calculated from the initial slope of the progress curve. If there was a lag phase, the activity was calculated after this phase. The activity was expressed as nanomoles of oxygen consumed per second (nkat).

Study of inhibition by halides ions was performed at pH 5 with 4-methylcatechol concentrations varying between 10 and 0.4 mM, in the absence and in the presence of inhibitors at three different concentrations. For the study of the influence of pH, inhibitor concentration that was close to the corresponding K_i value at pH 5 and the same range of concentrations for the substrate was used. Assays were performed in duplicate.

K_m and V_{max} values were determinated using a non-linear regression data analysis program for IBM PC (Sigma Plot, Jandel Scientific, Erkrath, Germany).

RESULTS AND DISCUSSION

Figure 1 shows the inhibitory effect of chloride on palmito PPO. From this plot it was deduced that chloride was a non-competitive mixed type inhibitor (K_m and V_m are modified in the presence of the ion). The co-ordinates of the points of interception are given by the relationship:

$$\frac{1}{[S]} = -\frac{1}{(\alpha K_S)}$$

and

$$\frac{1}{V} = \frac{1}{V_{max}} \frac{\alpha - 1}{\alpha},$$

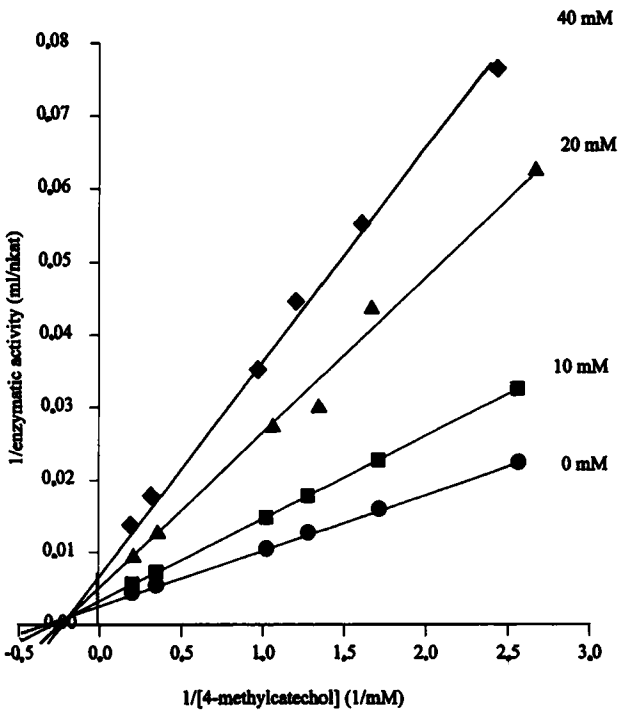


FIGURE 1 Inhibition of palmito PPO by sodium chloride. Oxygen consumption was measured by an oxymeter at 30°C and at pH 5 (McIlvaine buffer) with 4-methylcatechol as substrate.

where α is the factor that modified K_S when the inhibitor (I) binds to the enzyme before the substrate–enzyme complex is formed. For a non-competitive mixed type inhibition[10] $\alpha > 1$. The same type of inhibition was observed with fluoride ion. As far as bromide and iodide were concerned the inhibition was non-competitive.

The previously observed inhibition by NaCl and NaF showed that these salts have either a simple competitive or non-competitive type inhibitory effect; NaCl was found to be a competitive inhibitor of tyrosinase from frog epidermis,[5] NaF exhibited a competitive inhibitory effect on apple PPO while a non-competitive inhibition pattern was observed with NaCl.[8] Palmito PPO apparently exhibits a different behaviour towards these two halides salts.

The inhibition constants, K_i, and α values are shown in Table I. F^- was found to be the most effective inhibitor with a K_i value of 0.41 mM. Hence, the inhibitory power of the different halide salts on palmito PPO is $F^- > I^- = Br^- > Cl^-$. With the Brazilian palmito PPO, 50% inhibition was observed with 0.7 M NaCl and 3.5 M NaF.[11] The inhibition potency pattern observed by Janovitz-Klapp *et al.*[8] with apple PPO showed that F^- was the strongest inhibitor ($K_i = 0.07$ mM) followed by Cl^- (20 mM) and I^- and Br^- (0.11 and 0.12 M respectively). In the case of tyrosinase from frog epidermis, the inhibitory power pattern was $I^- > Br^- > Cl^- > F^-$.

Inhibition by halide salts could involve an action of the halide ion on the copper atom of the active site. Halide ions were reported to form a complex with copper in tyrosinase from *Neurospora*.[12] The classification of halides with regard to their inhibitory power can be indicative of the accessibility of the catalytic site.[10]

In the case of palmito PPO, inhibition occurs in the order $F^- > I^- = Br^- > Cl^-$. However the relative size of these ions is $F^- < Cl^- < I^- < Br^-$. Therefore, inhibition of PPO by halide ions could result from the conjugation of two opposite effects: the interaction forces between the halide ion and copper and the poor accessibility of the catalytic site for bulky ions.

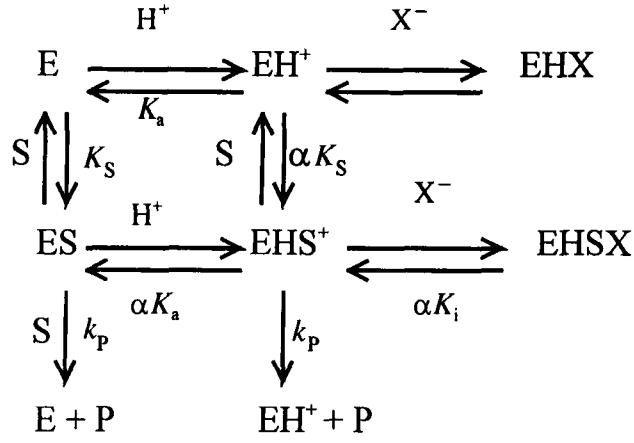
TABLE I Kinetic constants for inhibition of palmito PPO by halide ions

Inhibitor	*Type of inhibition*	K_i (mM)	α
NaCl	mixed	16.3	1.3
NaF	mixed	0.41	2.8
NaBr	non-competitive	9.15	—
NaI	non-competitive	8.59	—

(—): not determined.

Influence of pH on Inhibition by Fluoride and Chloride Ions

The influence of pH on the inhibitory effects of sodium fluoride and sodium chloride on palmito PPO was investigated. Inhibition by these halide ions showed to be strongly pH-dependent. The more acidic was the pH, the more pronounced was the inhibitory effect. The type of inhibition was unchanged irrespective of pH; a mixed type inhibition was found over the pH studied with both fluoride and chloride (Table II). The effects of pH on inhibition of PPO by halide salts have been studied in apple[8] and in frog epidermis.[5] The latter showed that chloride ions compete with a ligand for the copper atom and displace the ligand. The ligand involved would be a histidyl residue.[13] The mode of action of fluoride ion in palmito PPO would be similar to chloride ion since both Cl^- and F^- have similar inhibitory effects. Hence it is suggested that the mechanism of inhibition of palmito PPO by halides ions would be similar to that proposed by Penafiel *et al.*[5] for the inhibitory effect of chloride ion on frog tyrosinase. Results obtained by the latter showed a competitive type of inhibition; chloride ion would compete with the substrate when the enzyme is in the protonated form. In the present study, both halide ions showed a mixed type inhibition. It is therefore suggested that Cl^- ion (or F^-) binds to the protonated free enzyme (EH) or the protonated enzyme–substrate complex (EHS) and that the resulting forms EH–Cl (or EH–F) and EHS–Cl (or EHS–F) do not lead to the transformation of the substrate into the corresponding products. The inhibition scheme proposed by Penafiel *et al.*[5] can therefore be completed as follows:



with *X* as halide ion.

TABLE II

pH		3.6	4	4.56	5
without inhibitor	K_m(mM)	5.23	3.75	4.51	2.63
	V_{max}(nkat/ml)	112.7	217.1	226.2	245.6
NaF 0.2 mM	K_m(mM)	8.95	7.01	4.77	3.44
	V_{max}(nkat/ml)	60.1	99.8	150.3	162.1
	K_i(mM)	0.021	0.069	0.095	0.22
	α	9.45	6.24	2.95	1.95
NaCl 20 mM	K_m(mM)	6.51	5.26	4.55	2.98
	V_{max} (nkat/ml)	109.8	142.9	158.1	167.3
	K_i(mM)	17.26	14.16	21.145	26.38
	α	1.68	6.88	7.51	1.37

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