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Peroxidase and Chilling Injury in Banana Fruit

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Storage of unripe banana fruit (*Musa sapientum*) at 10 °C lead to development of chilling injury. The chill-injured fruit showed low peroxidase activity as compared to fruit stored at normal ripening temperature (healthy). Polyacrylamide gel electrophoretic analysis showed that the isozyme pattern for peroxidase was different for chill-injured and healthy fruit. Studies with partially purified chill-injured and healthy enzyme suggest that peroxidase may be one of the enzymes affected during low-temperature storage.

Temperature is one of the most important environmental condition that affects the ripening process. When fruits are stored below a certain critical temperature, the process of normal ripening does not occur. Instead, the fruits develop "chilling injury", a physiological abnormality that manifests with symptoms such as lack of flavor, lack of taste, and delayed ripening (Murata, 1969).

Haard (1973) observed that during ripening of banana there was an increase in the particulate (60000*g* pellet) peroxidase. This fraction was identified as "intracellular bound peroxidase". The soluble (60000*g* supernatant) and wall-bound peroxidases (solubilized from 60000*g* pellet by 0.1–0.2 M $CaCl_2$) were found to be invariant during normal ripening. During chilling injury the soluble and wall-bound peroxidases increased after 10–15 days of storage, whereas intracellular bound peroxidase exhibited negligible change (Haard and Timbie, 1973). The increase in soluble and wall-bound peroxidases after prolonged storage was viewed as a "hardening" effect.

In the present investigation we observed that during normal ripening of banana fruit the soluble (20000*g* supernant) peroxidase activity increased considerably as the fruit ripened. Since peroxidases are also implicated in cold adaptation of hardy plant tissues (McGown et al., 1969), we investigated the effect of low-temperature storage of banana fruit on the soluble peroxidase activity.

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MATERIALS AND METHODS

The unripe banana fruit were given ethylene treatment (10 ppm for 1 h) and subsequently stored at room temperature (28 ± 1 °C) and at 10 °C. The relative humidity was between 85 and 90% at both the temperatures. The fruits were sampled on different days, and their respiratory rate was measured as described earlier (Mattoo and Modi, 1975).

Enzyme Extraction and Assay. Peeled bananas were frozen at −15 °C and were ground in a mortar and pestle to form a pulp. PVP (1 g/2 g of pulp) was then added followed by twice the volume of 0.05 M PO_4 buffer, pH 7.2, to make a 50% homogenate. The homogenate was centrifugated at 20000*g* for 20 min at 4 °C. The resultant supernantant was taken as the soluble fraction and used for enzyme assay.

The peroxidase was assayed by Worthington's method ("The Worthington Manual", 1963). One unit of peroxidase activity is the amount of enzyme that catalyzes the conversion of 1 μmol of peroxide/min under the given assay conditions. Indole-3-acetic acid (IAA) oxidase was assayed by method described by Meudt and Gaines (1967). One unit of IAA oxidase activity is the amount of enzyme that degrades 1 μmol of IAA/min under the given assay conditions.

Protein in the cell-free extracts was estimated by analysis of 10% trichloroacetic acid (TCA) precipitates of

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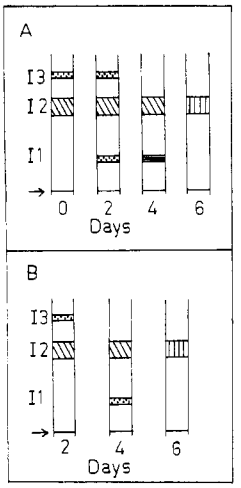

Figure 1. Electrophoretic separation of peroxidase isozymes of banana pulp from (A) fruits stored at 28 °C and (B) fruits stored at 10 °C. The arrow indicates the position of the tracking dye. Relative activity of zones: ■ > ▨ > ▥ > ▤.

the extracts by the method of Lowry et al. (1951).

Polyacrylamide Gel Electrophoresis. Soluble proteins in extracts of fruit stored at 10 and 28 °C were resolved by electrophoresis in 7.5% polyacrylamide gel as described by Davis (1964) (Figure 1). The gels were electrophoresed at 8–10 °C with constant current of 3 mA/tube for about 2.5 h. About 100 μg of protein of each sample was loaded on polyacrylamide gel. The dye front was allowed to migrate until it reached the lower end of the gel. Peroxidase bands were located by staining the gel in 0.1 M sodium acetate buffer, pH 5, containing 3% H_2O_2 and 1% *o*-dianisidine.

Enzyme Purification. Peroxidase in fruit stored at 28 and 10 °C for 4 days were purified. The enzyme was extracted as described above. The extracts were fractionated by $(NH_4)_2SO_4$ precipitation. Peroxidase activity in extracts of healthy fruit precipitated at 25–75% $(NH_4)_2SO_4$ whereas activity from chill-injured fruit precipitated between 50 and 90% $(NH_4)_2SO_4$.

The precipitate was resuspended in extraction buffer, dialyzed overnight against the same buffer, and loaded onto a Sephadex G-100 column (column size: 36 cm × 1.5 cm). The fractions (3 mL each) then were collected and assayed for the enzyme. The enzyme from chill-injured and healthy bananas was purified about 15- and 10-fold, respectively.

When palmitic acid was used, it was dissolved in a known volume of acetone before use. This volume of acetone had no effect on enzyme activity in control experiments.

RESULTS AND DISCUSSION

The onset of ripening in the "Climacteric" class of fruits is marked by an upsurge in the rate of respiration referred to as "respiratory climacteric or climacteric peak". This climacteric pattern of respiration is closely linked with the onset of ripening (Biale and Young, 1982). Because bananas are a climacteric fruit, the effect of low-temperature storage on the ripening process was studied by measuring the respiration rate. The fruits held at 28 °C showed the characteristic "climacteric" peak in respiration (1.08 g of CO_2 kg^{-1}/h^{-1}) after 6 days of storage. The respiration rate of bananas stored at 10 °C was about 42% lower at the climacteric peak (6 days) in comparison with the fruits stored at 28 °C.

After 3–4 days of storage at 10 °C, the skin was pale and the fruits failed to ripen normally when transferred to 28 °C. After about 8–10 days of storage at 10 °C, the central placenta became brown and hard. The fruits stored at 28 °C ripened normally after 8–9 days.

Table I. Peroxidase Activity of Banana Pulp during Storage at 28 and 10 °C[a]

time, days	bananas stored at 28 °C	bananas stored at 10 °C
0 (unripe)	1.10 ± 0.42	1.10 ± 0.42
2	2.07 ± 0.56	1.61 ± 0.36
4	3.66 ± 0.09	2.61 ± 0.11
6	3.68 ± 0.18	2.68 ± 0.47
8	3.74 ± 0.55	2.71 ± 0.25
10	4.01 ± 0.18	2.83 ± 0.12

[a] Units per milligram of protein. Each value is the mean ±SD of two replications from each of two separate experiments.

Table II. Magnitudes of Peroxidase Isozyme from Banana Pulp during Storage at 28 and 10 °C

	bananas stored at 28 °C			bananas stored at 10 °C		
time, days	I3[a]	I2	I1	I3	I2	I1
0 (unripe)	+	+++	-	+	+++	-
2	+	+++	+	+	+++	-
4	-	+++	++++	-	+++	+
6	-	++	-	-	++	-

[a] I = isozyme. The isozymes are numbered with decreasing electrophoretic mobility (I3 < I2 < I1). The profile is representative of one experiment and is typical of two other experiments.

Earlier studies on ripening of mango fruit implicated peroxidase as one of the key regulatory enzymes of the ripening process (Mattoo and Modi, 1975). In the present investigation, soluble peroxidase activity increased as the fruit ripened (Table I). However, storage at 10 °C slowed the increase in peroxidase activity (Table I).

Although Haard and Tobin (1971) found that casein dispersion was more effective than PVP for extraction of peroxidase from banana pulp, in the present study appreciable peroxidase activity is obtained by using PVP. The differences in the results could be due to the manner in which PVP is used. Also, we preferred PVP over casein since specific activity for the enzyme could be obtained and the purification of the enzyme in terms of *x*-fold purification could be calculated.

After 4 days of storage, peroxidase activity of fruit stored at 10 °C was about 30% less than the activity of fruit stored at 28 °C. A decrease in isocitratase activity has been reported (Mohapatra et al., 1970) for germinating cotton seedlings (about 43%) and peanut seedlings (about 18%) during exposure to chilling temperature for 6 h prior to harvest. The exposure of corn roots to cold shock resulted in changes in membrane permeability as evidenced by the ability of the roots to absorb 20–24% more Ca^{2+} than control roots (Zocchi and Hanson, 1982). Similarly, the mechanical injury of corn roots showed a 15–20% decline in adenine nucleotide content for the first hour after excision (Gronewald and Hanson, 1982). Thus, the 30% decrease in peroxidase activity at 10 °C was of significance for further study.

Polyacrylamide gel electrophoresis of banana pulp extract, from fruit stored at 28 and 10 °C, revealed the presence of two isozymes at unripe stage, with the appearance of another isozyme during ripening at 28 °C (Table II). This new isozyme species appeared 2 days later in 10 °C stored fruit. In addition, the amount of this isozyme in 10 °C stored fruit was significantly less as judged by the intensity of the enzyme activity band.

The substrate saturation kinetics were studied with partially purified preparation of peroxidase from healthy

Table III. Indole-3-acetic Acid Oxidase Activity of Partially Purified Enzyme from Healthy and Chill-Injured Bananas[a]

IAA concn, μM	healthy			chill injured		
	30 °C (a)	10 °C (b)	b/a × 100, %	30 °C (c)	10 °C (d)	d/c × 100, %
10	3.6	0.34	9	0.31		
30	11.32	1.54	14	1.23	0.10	8
50	17.14	4.63	27	5.83	0.68	12
90	18.51	7.54	40	5.73	0.92	16

[a] Units per milligram of protein at 30 and 10 °C.

and chill-injured bananas. The apparent K_m for the chill-injured enzyme preparation was 2.5 mM and for healthy bananas 1.5 mM. Thus, the enzyme from chill-injured banana has a lower affinity toward H_2O_2 than the enzyme from healthy bananas.

Indole-3-acetic acid (IAA) retarded ripening at low concentrations, whereas it promoted ripening at higher concentration (Tingwa and Young, 1975). IAA also retarded ripening in banana (Vendrell, 1969) and pear (Frenkel and Dyck, 1973). IAA oxidase activity developed during ripening of tomato, pear, and blueberry (Frenkel, 1972). Peroxidase-catalyzed degradation of IAA may be an important initiator of the ripening process. However, it may also be a consequence of the ripening process.

The IAA oxidase activity of bananas stored for 4 days at 28 and 10 °C was assayed at 10 and 30 °C (Table III). Since bananas develop chilling injury at 10 °C, the in vitro measurement of activity at 10 °C would give some information about the ability of the enzyme to degrade IAA during storage at 10 °C. The results indicate that the healthy banana enzyme was 9–40% active at an assay temperature of 10 °C in comparison with its activity at 30 °C for IAA concentrations of 10–90 μM. The chill-injured banana enzyme, in contrast, showed either no activity (at an IAA concentration of 10 μM) or a maximum of 16% activity (above a saturating IAA concentration of 50 μM) at an assay temperature of 10 °C as compared to its activity at an assay temperature of 30 °C. Thus, at the saturation level of IAA (50 μM), the healthy fruit enzyme had 3 times more IAA oxidase activity than chill-injured fruit enzyme at an assay temperature of 30 °C. However, at an assay temperature of 10 °C, the healthy fruit enzyme had 8 times more IAA oxidase activity than chill-injured fruit enzyme at 50 μM IAA.

Mango peroxidase was stimulated by palmitic acid (Mattoo and Modi, 1975). Palmitic acid did not have any significant effect on the chill-injured banana enzyme, but the enzyme from healthy bananas was stimulated 20% by 20 μM palmitic acid. The mango peroxidase preparation was stimulated 18% by 17 μM palmitic acid (Mattoo and Modi, 1975).

The optimum pH for enzyme activity was 5 for both chill-injured and healthy preparations. The chill-injured enzyme was more heat stable than the healthy enzyme (data not provided).

Demonstration that the enzyme from chill-injured bananas has less affinity for H_2O_2, oxidizes IAA at a slower rate, and is not affected by palmitic acid compared to enzyme from healthy bananas suggests that these activities may be functions of the isozyme that appears during ripening and is present at low levels in chill-injured tissue. Studies on the isolated isozyme may support this suggestion. The data show that peroxidase is one of the enzymes in the ripening banana that is affected during low-temperature storage.

Registry No. IAA oxidase, 9027-85-4; peroxidase, 9003-99-0.

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