Biochimica et Biophysica Acta, 397 (1975) 318—330

BBA 67577

PALMITIC ACID ACTIVATION OF PEROXIDASE AND ITS POSSIBLE SIGNIFICANCE IN MANGO RIPENING

A.K. MATTOO and V.V. MODI

Department of Microbiology, M.S. University of Baroda, Baroda 390002 (India)

(Received February 4th, 1975)

Summary

Palmitic acid stimulated the activity of mango peroxidase and reversed the inhibition due to the peroxidase inhibitor present in the preclimacteric fruit. The palmitic acid effect appeared to saturate in the range of 45 to 60 μM palmitic acid. Crude fatty acid extract of the mango exerted similar effect. The percentage stimulation was pH-dependent. Palmitic acid stimulated the enzyme by 18 percent at its optimum pH (5) but the stimulation was in excess of 63 percent at pH 2.5. At pH 2.5 the enzyme concentration versus velocity plot was non-linear and the activation by palmitic acid appeared to saturate between 32 and 48 μM concentration of the effector. The inhibition of the enzyme at and above 0.86 mM concentration of substrate (H_2O_2) was not found in the presence of palmitic acid. The effector also changed the heat inactivation kinetics of the enzyme and activated only two out of the four peroxidase isoenzymes present in the climacteric fruit extracts. The results presented indicate the regulatory nature of the enzyme and support its significance in fruit ripening.

Introduction

Although peroxidase (Donor:H_2O_2 oxidoreductase, EC 1.11.1.7) is widely distributed in plant cells [1—3] and has been crystallized from a number of sources [3,4] its physiological role is still largely unknown. Reports on the involvement of peroxidase or peroxidase-like enzymes in fruit ripening have appeared [5—8]. Mattoo et al. [9] have reported that a considerable increase in peroxidase occurs at the climacteric in mango (*Mangifera indica*) fruit and that the preclimacteric fruit contains natural proteinaceous inhibitor of this enzyme [10]. Also it has been shown that treatment of preclimacteric fruit slices with ethylene, the ripening hormone, results in stimulation of peroxidase and inac-

tivation of the proteinic inhibitor in the treated slices.

The experiments described in this paper show that physiological levels of fatty acids, in particular palmitic acid, stimulate the activity of mango peroxidase and reverse the inhibition that is caused by the addition of peroxidase inhibitor present in the preclimacteric fruit. Evidence has been presented which indicates the regulatory nature of this enzyme.

Materials and Methods

Mangoes. Alphonso mangoes [10] were used in the present investigation.

Enzymes and preparation of enzyme extracts. Horseradish peroxidase was purchased from Sigma Chemical Co., St. Louis, Mo. Methods employed for the preparation of cell-free extracts, estimation of peroxidase activity and determination of protein content in enzyme preparations were essentially the same as described earlier [9].

Polyacrylamide gel electrophoresis. The soluble proteins of fruit harvested at successive stages of ripening were resolved by subjecting the fruit extract to electrophoresis in 7.5% acrylamide gel as described by Davis [12]. Electrophoresis was carried out at 5°C using 2.5 mA per tube in 0.1 M glycine/NaOH buffer (pH 8.4) for 2 h. Peroxidase bands were located in reference gels by staining by dipping the gels first into 0.1 M sodium acetate buffer (pH 5) containing 3% H_2O_2 and then into either 1% *O*-dianisidine or 20 mM guaiacol.

For the elution of enzyme fractions the gel columns were removed from the tubes, frozen and sliced into 5 mm sections. Each section was extracted with 2 ml of 0.2 M Tris · HCl buffer (pH 7.4) containing 0.1 M KCl, with constant shaking at 5°C overnight.

Determination of respiration (CO_2 evolved). All the operations were carried out at 25°C. Fresh preclimacteric (unripe) fruits, weighing about 300—400 g, were placed in air-tight containers, having two way connections. Moisture-free and CO_2-free stream of air was passed through these containers, over the mangoes, and the CO_2 evolved was collected in an air-tight flask containing 1 M KOH. The relative humidity in the containers was maintained at 55—65%. The collection was carried out for 2—3 h each day. For corrections, the air (free of moisture and CO_2) was passed through the containers before placing the fruits in them. After the collection was over the flasks containing 1 M KOH and the absorbed CO_2 were disconnected and to them a saturated solution of barium chloride was added to precipitate out barium carbonate. After filtration, the filtrate was titrated against 1 M HCl using methyl red as an indicator, for the determination of residual KOH. CO_2 evolved in terms of ml per h per kg fresh fruit was calculated. The experiments were run till the unripe fruits reached the senescence stage.

Preparation of total lipids and crude fatty acids. Total lipids and crude fatty acid mixture of fruit pulp were prepared by the procedure reported earlier [13].

The effectors used namely, fatty acid mixture and palmitic acid were dissolved in a known volume of acetone before use. The amount of acetone added in the control tubes (= 0.05 ml) was found to have no effect on the enzyme activity.

Results

Mattoo and Modi [9,10] have reported that peroxidase from climacteric mango is inhibited by an inhibitor present in the preclimacteric fruit. The inhibitor was partially characterized as a protein. The results in Fig. 1 illustrate time course change in the inhibition of peroxidase activity caused by its inhibitor and reversal of this inhibition by palmitic acid at pH 5 and temperature 25° C. Palmitic acid was found to stimulate the control reaction by two folds (Table I). From the Fig. 1 it is clear that in the presence of fruit inhibitor there is considerable decrease and an initial lag in the activity of peroxidase; inclusion of palmitic acid in the inhibited enzyme system results in reducing the lag period and restoring the enzyme activity. The activation by palmitic acid is essentially instantaneous and preincubation with palmitic acid is not required in order to observe maximal activity.

The effect of varying enzyme concentrations on the enzyme activity in the absence and the presence of inhibitor, and inhibitor and palmitic acid, is depicted in Fig. 2. At all the concentrations of the enzyme protein palmitic acid was found to overcome the inhibition caused by the peroxidase inhibitor. The nature of peroxidase activation by palmitic acid was further explored.

Results listed in Table II show that the activation of peroxidase activity increases with increasing concentrations of palmitic acid. The maximal activation (60%) observed in this experiment occurred on addition of 50 μM palmitic acid. The effect appears to saturate in the range of 45 to 60 μM of palmitic

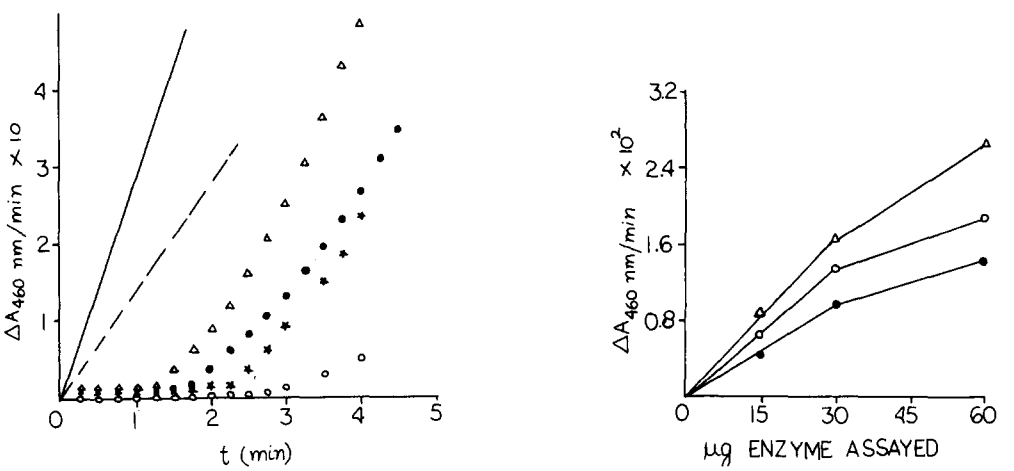


Fig. 1. A typical time course change in mango peroxidase in the presence of inhibitor in the absence and presence of palmitic acid. The enzyme system and preincubation conditions were those described in Table I. Inhibitor concentration, when added, was 30 μg per system. When palmitic acid was included in the system its concentration was 27 μM. The pH of assay was 5. ○———○, enzyme preincubated with the inhibitor before assay; ●———●, palmitic acid added to the inhibited system at 90 s; *———*, inhibitor added to the enzyme assay mixture at zero min (no preincubation); △———△, palmitic acid added to the inhibited enzyme system (preincubated system) at zero min of assay. Broken line indicates the control enzyme activity and the unbroken line indicates the enzyme activity in the presence of palmitic acid.

Fig. 2. Effect of assaying various enzyme concentrations from the preincubated enzyme systems at pH 5.0. Details of preincubation system are given in Table I and Fig. 1. ●———●, enzyme plus inhibitor (38 μg); ○———○, enzyme alone; △———△, enzyme plus inhibitor (38 μg) plus palmitic acid (27 μM).

TABLE I

INHIBITION OF MANGO PEROXIDASE BY PROTEINIC INHIBITOR AND REACTIVATION EFFECT OF PALMITIC ACID

The preincubation mixture contained 0.1 M Tris · HCl (pH 7.2) and enzyme. Proteinaceous inhibitor was added where indicated. After 15 min at 0°C, an aliquot of each tube was added to the assay mixture. The assay mixture contained in a total volume of 3 ml, 0.833 mM H_2O_2, 10 mM sodium acetate buffer (pH 5) and 25 µl *O*-dianisidine (1% in methanol). Inhibitor and palmitic acid were added where indicated. No loss of activity was found in the control enzyme after incubation. The figures are taken from the linear portions of the curves in Fig. 1.

Addition to preincubation mixture	Addition to assay mixture	$\Delta A_{460\,nm}$/min
—	—	0.135
—	Inhibitor (30 µg)	0.060
Inhibitor (30 µg)	—	0.012
Inhibitor (30 µg)	Palmitic acid (27 µM)	0.121
—	Palmitic acid (27 µM)	0.270

TABLE II

EFFECT OF PALMITIC ACID AND CRUDE FATTY ACID MIXTURE ON MANGO PEROXIDASE ACTIVITY

The assay system was the same as described in Table I. One unit corresponds to one µmol H_2O_2 oxidised min^{-1} at 25°C. The enzyme concentration was 50 µg per system. The concentrations of crude fatty acid mixture mentioned are with respect to palmitic acid.

Effector	Peroxidase (units/mg protein)	% Activation
—	0.0265	—
Palmitic acid (16.6 µM)	0.0322	21
Palmitic acid (33.2 µM)	0.0414	56
Palmitic acid (50.0 µM)	0.0425	60
Crude fatty acid mixture (16.6 µM)	0.0364	35
Crude fatty acid mixture (33.2 µM)	0.0375	41
Crude fatty acid mixture (50.0 µM)	0.0427	61

TABLE III

EFFECT OF PALMITIC ACID AND MANGO FATTY ACID MIXTURE ON HORSERADISH PEROXIDASE ACTIVITY

The assay system was same as in Table I. Horseradish enzyme concentration in the system was 1 µg. The concentrations of crude fatty acid mixture mentioned are with respect to palmitic acid.

Effector	Horseradish peroxidase $\Delta A_{460\,nm}$/min	% Activation
—	0.309	—
Palmitic acid (8.3 µM)	0.386	24.8
Palmitic acid (16.6 µM)	0.428	38.5
Crude fatty acid mixture (83 µM)	0.387	25.0
Crude fatty acid mixture (166 µM)	0.510	65.0

acid; a decrease in the activation of peroxidase occurred beyond 60 μM palmitic acid. Crude fatty acid extract of the mango exerts the same effect (Table II) thus demonstrating that a naturally occurring lipid product from the fruit could be of some physiological significance in the control of the activity of this enzyme. No attempt was made to quantitate the fatty acid extract. Qualitatively, however, the extract was found to contain palmitate, oleate, linoleic and linolenate. Similar activation of horseradish peroxidase by palmitic acid and crude fruit fatty acids was also observed (Table III).

The enhancement by palmitic acid could result from a shift in the pH optimum of the peroxidase. The profiles for the enzyme assay in the presence and the absence of palmitic acid as a function of pH are illustrated in Fig. 3. No detectable shift in the pH optimum of the peroxidase was observed when palmitic acid was added. In each case the activity decreased with decreasing pH but the activity in the absence of palmitic acid dropped more rapidly. Thus at lower pH values a greater percentage stimulation was observed. Near the optimal pH (5) of the mango enzyme palmitic acid stimulated less than 18% whereas

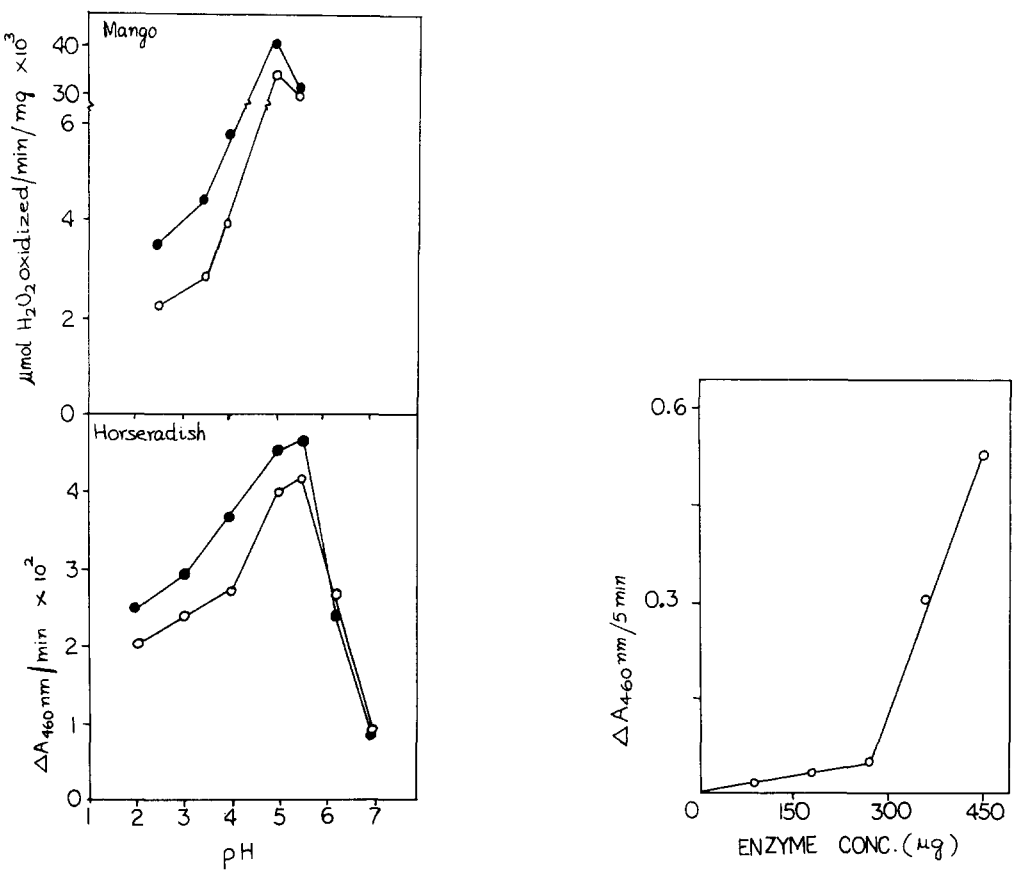


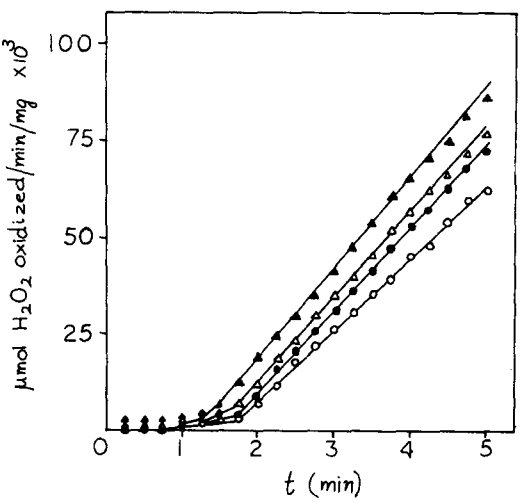
Fig. 3. Effect of pH on the activation of mango and horseradish peroxidase in the absence and presence of 17 μM palmitic acid. Mango peroxidase used was 50 μg protein and horseradish enzyme was 1 μg. ○———○, control enzyme; ●———●, enzyme plus palmitic acid.

Fig. 4. The relation of enzyme protein to peroxidase activity at pH 2.5. See the text for details.

at pH 2.5 a stimulation in excess of 63% was observed. Similar situation was observed in horseradish peroxidase at pH 4 and pH 6 (Fig. 3). During this experiment it was found that at pH 2.5 there was much less activity with a considerable lag in the activity of fruit enzyme when the concentration of the enzyme was in the range of 60 to 270 μg per system. This lag period decreased effectively when the concentration of the enzyme exceeded 270 μg per system, resulting in an upsurge of enzyme activity. Velocities which were a linear function of time of incubation were compiled and relation between the enzyme concentration and the activity of mango peroxidase is represented in Fig. 4. It is evident that at pH 2.5 the enzyme concentration vs velocity is non-linear. This could happen either due to the buffering offered by more protein or due to changes in the aggregation of enzyme molecules.

Observation of a higher percentage stimulation by palmitic acid at pH 2.5 appears to be of significance in view of the fact that pH of the mango changes from 2 in the preclimacteric fruit to 5 in the climacteric fruit. The effect of varying concentrations of palmitic acid at pH 2.5 as a function of time is depicted in Fig. 5. The enzyme concentration used was 360 μg of protein. With an increase in palmitic acid from 16 to 48 μM there is a relative increase in the enzyme activity and some reduction in the initial lag observed in the absence of palmitic acid. The effect appears to saturate in the range of 32 to 48 μM as further increase in palmitic acid yielded no more activation.

The heat inactivation kinetics for peroxidase in the absence and the presence of palmitic acid were studied at 50°C. Inactivation of the enzyme under both the conditions seems to be exponential but non-linear (Fig. 6). The initial rate of inactivation (up to 3 min) in the enzyme incubated with palmitic acid is slow as compared to that for the enzyme incubated without the effector. With



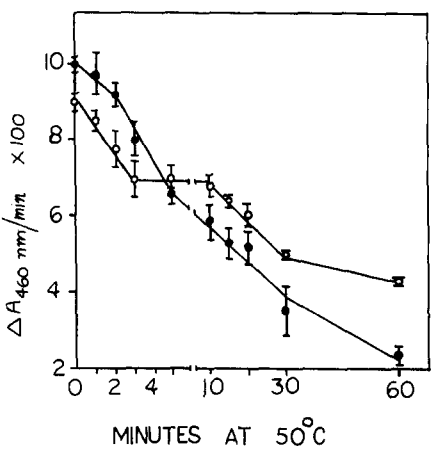


Fig. 5. Time course change in peroxidase activity at pH 2.5 in the presence of various fixed concentrations of palmitic acid. The enzyme concentration was 360 μg per system. Palmitic acid: ○, nil; ●, 16 μM; △, 20 μM; ▲, 40 μM.

Fig. 6. Heat inactivation kinetics for peroxidase at 50°C. ○———○, control enzyme; ●- - -●, enzyme plus palmitic acid (0.4 mM). The pH was 5.8. Bars indicate the standard deviation of 5 experiments.

increase in the incubation time at 50°C the rate of inactivation is significantly faster in the enzyme incubated with palmitic acid than in that incubated without it. Within 60 min at 50°C the enzyme incubated with palmitic acid lost about 77% of the original activity whereas that incubated without it lost only about 52% activity. This dual behaviour of the enzyme indicates that palmitic acid induces some modifications in the enzyme in such a way that the response to heat inactivation changes markedly from the control enzyme.

To ascertain whether the activation of peroxidase is due to the binding of any inactivating metal by the chelating action of the fatty acid effect of various fixed concentrations of EDTA, 2-2 bipyridyl, sodium citrate and palmitic acid on mango peroxidase at pH 5 was studied. The results of this experiment, summarised in Table IV, indicate that the chelating agents inhibit the enzyme activity. Sodium citrate proved to be by far the most potent inhibitor. From the Table it is also evident that in the presence of 14 μM palmitic acid about 15% inhibition caused by 10 mM of sodium citrate is reversed. These results support the metalloenzymic nature of mango peroxidase and further rule out the possibility that palmitic acid activation is due to a chelating action.

The substrate saturation kinetics were studied with a partially purified preparation of mango peroxidase. (The precipitates obtained at 0—0.5 and 0.5—1.0 ammonium sulphate saturations of the cell-free extract were collected, suspended in a known volume of the buffer, pH 7.2, and dialysed against 0.01 M Tris · HCl buffer (pH 7.2) for 40 h. The suspension was then concentrated by dialysis against solid sucrose to one-seventh of the original volume and cleared of any suspending particles by centrifugation. About 8 mg of this concentrated and cleared fraction were applied to the top of a Sephadex-G 100 column (column size, 40 cm × 2 cm) previously equilibrated with 0.01 M Tris ·

TABLE IV

EFFECT OF CHELATING AGENTS AND PALMITIC ACID ON THE ACTIVITY OF MANGO PEROXIDASE

The assay system was same as in Table I. The results were compiled from the time course change curve vs absorbance and only those values which were a linear function of enzyme activity are included. Enzyme concentration was 50 μg per system.

Compounds	Peroxidase (units/mg protein)	% Change
—	0.120	—
EDTA (0.01 mM)	0.113	(−) 5.83
EDTA (0.1 mM)	0.113	(−) 5.83
EDTA (1.0 mM)	0.094	(−) 21.6
EDTA (10.0 mM)	0.082	(−) 31.6
Bipyridyl (0.01 mM)	0.127	(+) 5.83
Bipyridyl (0.1 mM)	0.120	—
Bipyridyl (5.0 mM)	0.094	(−) 21.6
Sodium citrate (5.0 mM)	0.042	(−) 65.0
Sodium citrate (10.0 mM)	0.037	(−) 69.0
Sodium citrate (50.0 mM)	0.009	(−) 92.5
Sodium citrate (100 mM)	0.000	(−) 100
Palmitic acid (7.0 μM)	0.144	(+) 20.0
Palmitic acid (14.0 μM)	0.156	(+) 30.0
Sodium citrate (10 mM) + palmitic acid (14 μM)	0.054	(−) 55.0

HCl buffer (pH 7.2). The filtration rate was maintained at 1 ml per 5 min and the effluent fractions were collected in 10 ml portions. Most of the peroxidase units came out in fraction V (peak I) and a second major fraction came out in fraction XVII. The fractions comprising Peak I were pooled, concentrated to about one-tenth of the original volume (by dialysis against sucrose), and dialysed for 5 h against distilled water. The V of this fraction was found to be 0.77 μmol · min^{-1} · mg^{-1} protein and that of the crude cell-free extract was 0.02 μmol · min^{-1} · mg^{-1} protein, K_m did not change. This way about 39 fold purification was achieved).

The effect of constant amounts of palmitic acid and purified enzyme fraction on different concentrations of the substrate, H_2O_2, is illustrated in Fig. 7. In the absence of palmitic acid, the substrate velocity plot of H_2O_2 oxidation by purified enzyme fraction was slightly sigmoidal at low substrate levels (under 0.2 mM) and hyperbolic at higher levels (up to 0.83 mM). There was a clear indication of a substrate-inhibition of the enzyme after 0.86 mM substrate level (Figs 7a and 7b). Significantly, the presence of palmitic acid

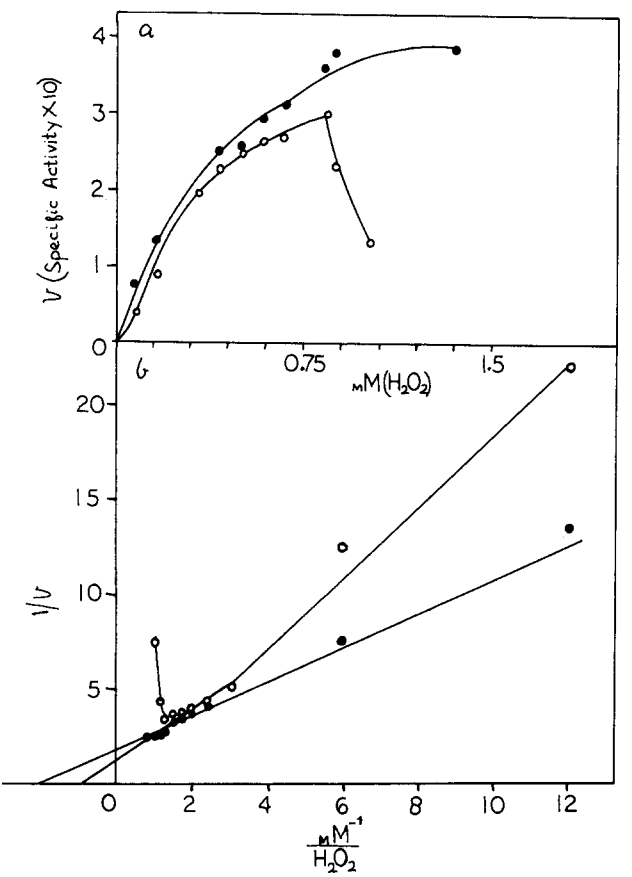


Fig. 7. (a) Substrate saturation kinetics of mango peroxidase with H_2O_2 concentrations ranging from 0.083 to 1.33 mM. The pH was 5.0 and the protein concentration of the partially purified preparation was 9 μg per system. ○———○, no palmitic acid; ●———●, with palmitic acid (16.6 μM). (b) Lineweaver-Burk plot of the data in (a).

reduces the sigmoidal tendency and substrate-inhibition character of the curve relating substrate level to reaction velocity and the curve tends to approach conventional Michaelis Menten kinetics. Extrapolation of the linear portions of the curves (i.e. values corresponding to high substrate concentrations) gave the values for the apparent Michaelis constant. The apparent K_m for H_2O_2 in the absence of palmitic acid was 1.11 mM; in the presence of palmitic acid the K_m was 0.5 mM. By lowering the K_m, palmitic acid increases the affinity of the enzyme for its substrate presumably through a change in the protein conformation which might explain in part the reversal of the inhibition of the enzyme reaction by the proteinic inhibitor (Fig. 1 and Table I). Palmitic acid was also found to lower the K_m of horseradish peroxidase for H_2O_2 by about 50%.

Changes in the activity of those enzymes which occur in multiple molecular forms (isoenzymes) can also result due to an effect either on all the isoenzymic fractions or more efficiently on a few specific ones depending upon their structural interrelations. Peroxidases are known to exist in multiple molecular forms [14—19]; some of the peroxidase isoenzymes even have been isolated and purified [14—16,20]. In order to explore further the significance of palmitic acid activation of peroxidase, it was felt necessary to determine which of the isoenzymes respond to palmitic acid.

Polyacrylamide gel electrophoresis of climacteric fruit extracts revealed consistently four peroxidase bands; isoenzymes PI, PII, PIII and PIV (Fig. 8). Very faint and inconsistent bands of isoenzymes PI and PII were detectable in the extracts prepared from preclimacteric fruit. Table V lists the specific activity of the four isoenzymes which were eluted from the gels. On storage at 0—4° C for 48 h the eluted fractions lost more than 50% activity, the maximum loss occurred in isoenzyme PIV (about 68%) indicating the unstable nature of the mango peroxidase isoenzymes (Table VI). Each isoenzyme was tested for enzymic activity in the presence of 40 μM of palmitic acid, the results of which

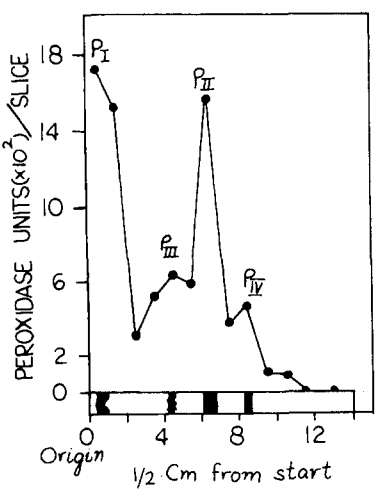


Fig. 8. Profile of peroxidase isoenzymes in climacteric fruit extract on polyacrylamide gel electrophoresis. The position of the isoenzymes on the gel are indicated in the horizontally drawn diagrammatic view of the gel. Details of the method used are given in Materials and Methods.

TABLE V

SPECIFIC ACTIVITY OF ELUTED PEROXIDASE ISOENZYMES BEFORE AND AFTER STORAGE

The method of the elution of isoenzymic fractions from the gels is described in Materials and Methods. The eluted fractions were preserved at 0—4°C.

Isoenzyme	Peroxidase activity (units per mg protein)		
	Fresh	After 48 h	% loss
PI	0.6137	0.2655	56.7
PII	0.1725	0.0700	59.4
PIII	0.2160	0.0896	58.5
PIV	0.0380	0.0123	67.6

are shown in Table VI. It was of interest to note that palmitic acid activated only isoenzymes PI and PII; isoenzymes PIII and PIV were not affected at all. To see if any other saturated fatty acid of approximately the same carbon chain length can mimic palmitic acid effect one experiment was run with myristic acid. Myristic acid (40 μM) was found to activate isoenzyme PIV also, in addition to isoenzymes PI and PII, suggesting that the effects of palmitic acid and myristic acid may be different. No further attempt was made to pursue this line of investigation.

It was of interest to study what changes occurred in total lipid content with climacteric rise as the fruit ripened. The results presented in Fig. 9 indicate a considerable increase in the total lipids during ripening, the lipid level reaching a peak prior to the climacteric maximum. The sudden fall in total lipids prior to the peak of climacterc may be of much regulatory consequence. The total fatty acid content per 100 g fresh mango pulp varies from 140—230 mg in the unripe to 600—900 mg in the ripe fruit [13], out of which about 15—30% accounts for free fatty acids. Moreover, at the climacteric and post-climacteric stages the content of free fatty acids was found to increase further. The concentrations of palmitic acid or fatty acid mixture used in the experiments described above evidently fall within the physiological levels. In this context, the observations of Hulme et al. [21] are of much concern. Using discs from preclimacteric apples and incubating them in solution for periods up

TABLE VI

EFFECT OF PALMITIC ACID AND MYRISTIC ACID ON PEROXIDASE ISOENZYMES OF CLIMACTERIC MANGO

The enzyme assay system was same as in Table I. The enzyme activity was tested with palmitic acid (40 μM) and myristic acid (40 μM) in the indicated isoenzymes after storage for 48 hr at 0—4°C. N.T., not tested.

Effector	Peroxidase activity (μmol H_2O_2 oxidised · min^{-1} · mg^{-1} protein)			
	PI	PII	PIII	PIV
—	0.2655	0.070	0.090	0.0123
Palmitic acid	0.3834	0.125	0.100	0.0102
Myristic acid	0.4203	0.131	N.T.	0.0230

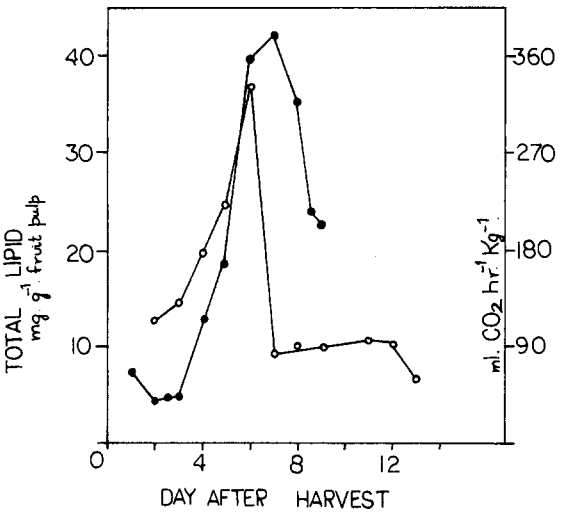


Fig. 9. Changes in total lipid content (○) and respiration (●) of mango fruit during its postharvest ripening. Details are given in the text.

to 24 h they suggested that an enhancement of turnover of lipid precedes the increase in the production of ethylene.

Discussion

In previous communications the involvement of peroxidase in mango metabolism has been established [9—11]. Using peroxidase as a model enzyme it was shown that proteinic compounds present in the preclimacteric fruit exert a negative control on this reaction [10]. The experiments described in Fig. 1 show that an additional control mechanism exists. That is, the inhibitory effect of proteinic inhibitor can be reversed by palmitic acid. The results described here provide some insight into the mechanism by which palmitic acid activates peroxidase, but do not permit a complete understanding of this phenomenon.

The binding of palmitic acid to the enzyme is rapid and does not cause a shift in the pH optimum (Fig. 3). The effect of pH on the mango peroxidase at low and high enzyme concentrations (Fig. 4), and in the absence and presence of palmitic acid (Fig. 3) reveal some interesting properties of the enzyme. These effects may be attributed to the prevention of the dissociation of the aggregated enzyme molecules at pH 2.5 and pH 3 by higher concentrations of enzyme protein and palmitic acid. Westhead [22] observed loss of helical regions of yeast enolase at acidic pH. Palmitic acid activation at pH 2.5, the pH of the preclimacteric fruit, appears to be of significance as it could happen in otherwise an unfavourable condition of low pH for enzyme activity.

The enzyme demonstrated complex kinetics (Fig. 7). The pronounced curvature, and substrate-inhibition, of the reciprocal kinetics plot particularly in the absence of palmitic acid suggests that the substrate itself (H_2O_2) functions as an effector [23]. The addition of palmitic acid decreases the prominence of the curvature and removes substrate inhibition. This may be related to the lack of co-operative kinetics for the substrate in the presence of palmitic

acid and indicates the existence of more than one binding site for substrates and the existence of the enzyme in more than one conformational state. From his results on the oxidation of indoleacetic acid by peroxidase enzyme, Meudt [24] has suggested the presence of two enzyme sites of horseradish peroxidase, each combining with a particular part of the indoleacetic acid molecule. Meudt also observed substrate-inhibition of indoleacetic acid oxidation and explained that at high substrate concentration indoleacetic acid molecules will compete for available enzyme sites resulting in the inhibition of enzymic oxidation of indoleacetic acid. A clear understanding of the stimulatory effect of palmitic acid obviously will depend on further purification and further study of the enzyme.

The results that palmitic acid increases the activity of the enzyme at low but not at high substrate concentrations, changes the heat inactivation kinetics of the enzyme (Fig. 6), activates only the two isoenzymes, PI and PII (Table VI), and that the effect is not just a chelation phenomenon (Table IV) point to the possibility of a conformational change of the enzyme protein and/or a focus of aggregation of several enzyme molecules in the presence of palmitic acid, thereby increasing the affinity of the active site for the substrate. Fatty acid, such as oleic acid, has been shown to induce conformational change in bovine serum albumin and result in additional strong binding sites [25].

A delicate substrate-inhibitor-activator balance or control is very possible for this enzyme in the intact cell, and as such the controlled activity of this enzyme could contribute substantially to the control of oxidative metabolism in a postharvest mango.

This paper also adds to the role that fatty acids play in the fruit metabolism. Earlier reports from this laboratory have shown that fatty acids may regulate the activity of the first two enzymes of the HMP shunt and malic enzyme by inhibiting these enzymes [26], and that fatty acids activate the citrate cleavage enzyme of the mango [13]. These data together with our results (Fig. 9) which indicate a sharp fall in total lipids prior to the climacteric rise in mango stress the significance of lipids and lipid components in fruit ripening and need a comprehensive study.

Acknowledgement

We thank Dr Morris Lieberman, Agricultural Research Centre, U.S. Department of Agriculture, Beltsville, Maryland (U.S.A.) for helpful suggestions and for going through first version of the manuscript.

References

1 Onslow, M.W. (1920) Biochem. J. 14, 535—540
2 Onslow, M.W. (1921) Biochem. J. 15, 113—117
3 Maehly, A.C. (1955) Method Enzymol. 2, 801—813
4 Saunders, B.C., Holmes-Seidle, A.G. and Stark, B.P. (1964) Peroxidase, Butterworths, London
5 Lieberman, M., Kunishi, A.T., Mapson, L.W. and Wardale, D.A. (1966) Plant Physiol. 41, 376—382
6 Ku, H.S., Yang, S.F. and Pratt, H.K. (1969) Phytochemistry 8, 567—573
7 Takeo, T. and Lieberman, M. (1969) Biochim. Biophys. Acta 178, 235—247
8 Sacher, J.A. (1973) Annu. Rev. Plant Physiol. 24, 197—224
9 Mattoo, A.K., Modi, V.V. and Reddy, V.V. (1968) Ind. J. Biochem. 5, 111—114
10 Mattoo, A.K. and Modi, V.V. (1970) Enzymologia 39, 237—247

11 Mattoo, A.K. and Modi, V.V. (1969) Plant Physiol. 44, 308—310
12 Davis, B.J. (1964) Ann. N. Y. Acad. Sci. 121, 404—427
13 Mattoo, A.K. and Modi, V.V. (1970) Biochem. Biophys. Res. Commun. 39, 895—904
14 Hagihara, B., Tagawa, K., Morikawa, I., Shin, M. and Okonuki, K. (1958) Nature 181, 1655—1656
15 Tagawa, K. and Shin, M. (1959) J. Biochem. Tokyo 46, 865—873
16 Shin, M. and Nakamura, W. (1961) J. Biochem. Tokyo 50, 500—507
17 Lanzani, G.A., Marchesini, A., Galante, E., Manzocchi, L.A. and Sequi, P. (1967) Enzymologia 33, 361—372
18 Haard, N.F. (1973) Phytochemistry 12, 555—560
19 Sequi, P., Marchesini, A. and Chershi, A. (1970) FEBS Symposium, Enzymes and Isoenzymes (Shugar, D., ed.) 18, 297—303
20 Morita, Y., Yoshida, C. and Maeda, Y. (1971) Agr. Biol. Chem. 35, 1074—1083
21 Hulme, A.C., Rhodes, M.J.C., Galliard, T. and Wooltorton, L.S.C. (1968) Plant Physiol. 43, 1154—1161
22 Westhead, E.W. (1964) Biochemistry 3, 1062—1068
23 Dixon, M. and Webb, E.C. (1958) Enzymes, p. 89, Academic Press, New York
24 Meudt, W.J. (1971) Phytochemistry 10, 2103—2109
25 Green, H.O., Moritz, J. and Lack, L. (1971) Biochim. Biophys. Acta 231, 550—552
26 Ghai, G. and Modi, V.V. (1970) Biochem. Biophys. Res. Commun. 41, 1088—1095