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Anions Activate the Oxidation of Indoleacetic Acid by Peroxidases from Tomato and Other Sources

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ABSTRACT

Anionic peroxidase from tomato (*Lycopersicon esculentum*) fruit oxidized indoleacetic acid (IAA) slowly in the presence of Mn^{2+} and dichlorophenol in acetate buffers. The addition of certain anions to the reaction mixture increased the rate of oxidation. Phosphate was one of the effective anions and exerted maximal activation at 0.1 molar. The most effective activator of tomato peroxidase was nitrilotriacetate (NTA) at an optimum concentration of 60 micromolar. Only 0.17 nanomolar peroxidase was needed to oxidize 0.1 micromole IAA/5 minutes in the presence of NTA compared to 650 nanomolar peroxidase for the same rate in the absence of NTA. Other effective anions were oxalate, pyrophosphate, malate, and citrate. Each activator exhibited an optimum concentration and higher concentrations were inhibitory. Anionic peroxidase from horseradish was activated by the same anions. A cationic peroxidase from horseradish and lactoperoxidase oxidized IAA in acetate buffer although anions activated these enzymes severalfold. Microperoxidase and other hematoporphrins also catalyzed IAA oxidation in the presence of anions. It is proposed that IAA oxidation by peroxidase may be important when vacuolar contents mix with peroxidase as during plant injury.

Peroxidase catalyzes a variety of reactions *in vitro* which may not have physiological functions in intact plants. One of these reactions is the oxidation of IAA in the presence of O_2, Mn^{2+}, and a monophenol (5, 11, 18, 27). Most peroxidases catalyze the oxidation but there have been reports of IAA oxidases without peroxidase activity (24, 25, 32) and of peroxidases that failed to oxidize IAA (12, 16). Isoenzymes from horseradish showed different ratios of peroxidatic/oxidatic activity (13). The information on tomato peroxidase as an IAA oxidase has been particularly conflicting. Kokkinakis and Brooks (14) measured the oxidation of IAA in citrate buffer and found that the tomato enzyme was much less effective than horseradish peroxidase. They subsequently reported that the oxidation by tomato peroxidase was enhanced by the inclusion of peroxide in the reaction mixture (15). The reaction rate decreased sharply when peroxide in the mixture was exhausted. Peroxide can reduce the lag period of IAA oxidation by other peroxidases, but its presence is not necessary to sustain the reaction (20). Furthermore, peroxide appears to interfere with promotive effects of Mn^{2+} in IAA oxidation by peroxidases (21, 30).

Huang and Haard (12) reported that IAA oxidase was detectable in extracts of tomatoes only after inhibitors of the reaction were removed. They also found that one or two IAA oxidases were obtained depending on extraction conditions. When tomatoes were extracted with water, two peroxidases were separated by gel filtration but only the larger enzyme oxidized IAA. Extraction of the same fruit with 0.2 M NaCl yielded the same peroxidases but both enzymes oxidized IAA. They observed that the effective peroxidases were optimally active at pH 6.1, or substantially higher than that for many other peroxidases (11, 24, 33). In the study of the effect of pH on the reaction, they used acetate buffer for the pH range of 4 to 6 and phosphate for higher pH. A possible explanation for the observed high pH optimum may be that the enzymes were more effective on IAA in phosphate than in acetate. Thomas and Jen (29) studied the oxidation of IAA by purified tomato peroxidase using phosphate buffer at pH 6. They confirmed that the enzyme was capable of oxidizing IAA in the presence of Mn^{2+} and DCP,[1] and that peroxide was not required.

During preliminary studies on the oxidation of IAA by tomato peroxidase, I observed that the rate of the reaction was dependent on the nature and concentration of the buffer. The reaction occurred very slowly in acetate at pH 3 to 6, but it proceeded rapidly when phosphate was included in the buffers. An examination of the reaction in other buffers led to the discovery that some anions have extraordinary effects on the oxidation of IAA by tomato peroxidase. The study was extended to oxidation of IAA by horseradish peroxidases and other porphyrin proteins.

MATERIALS AND METHODS

Purification of Tomato Peroxidase

One kg of pericarp tissue from green tomato (*Lycopersicon esculentum* cv Sweet Chelsea) fruit was homogenized with 1 L of 0.2 M sodium acetate (pH 6.0), containing 1.0 M NaCl. The homogenate was stirred for 1 h at 3 °C and centrifuged at 10,000*g* for 30 min. The supernatant solution was concentrated to 25 mL by ultrafiltration using a PM-10 membrane (Amicon Corp.) and dialyzed against 0.15 M NaCl. It was clarified by centrifugation and applied to a 5 × 40 cm column of Sephadex G-100 in 0.15 M NaCl. The fractions containing peroxidase were pooled, ultrafiltered to 20 mL and dialyzed against 0.05 M sodium acetate, pH 6.0 It was then applied to a 2.5 × 24 cm column of Q-Sepharose equilibrated with 0.05

[1] Abbreviations: DCP, dichlorophenol; NTA, nitrilotriacetate.

M sodium acetate (pH 6.0). Elution was conducted with 1 L of a linear gradient of 0.05 to 0.5 M NaCl. The fractions corresponding to the major peak of peroxidase were pooled, ultrafiltered to 2 mL and dialyzed against 0.02 M sodium acetate, pH 6.0. One-half mL aliquots of this solution were then chromatographed on a Mono Q column in a fast protein liquid chromatography system (Pharmacia). Elution was conducted with 30 mL of a linear gradient of 0 to 0.1 M NaCl containing 0.02 M sodium acetate (pH 6.0), at a flow rate of 1 mL/min. The fractions containing the major peak of peroxidase were combined, concentrated to 2 mL, and dialyzed against 0.02 M NaCl. This enzyme represents the main anionic peroxidase in tomato fruit (14, 29). The specific activity and R_Z value were 96,000 and 3.64, respectively.

Other Enzymes

An anionic isoenzyme from horseradish (type VIII, Sigma Chemical Co.) was purified by chromatography on a Mono Q column. Only the major peak of peroxidase was saved and it had a R_Z of 3.8. A cationic isoenzyme (type IX, Sigma Chemical Co.) was purified on a Mono S column to a R_Z value of 3.9. Lactoperoxidase, hemoglobin, myoglobin, Cyt *c*, catalase, and the microperoxidases MP-9 and MP-11 were obtained from Sigma Chemical Co. and used without further purification.

Peroxidase Assay

Peroxidase was measured in a reaction mixture containing 0.3 mL of 0.2 M Mes (pH 6.0), 0.15 mL of 0.16 M H_2O_2, 0.15 mL of 0.16 M quaiacol, and 2.3 mL of water. The solution was placed in a cuvette and 0.1 mL of enzyme solution was added at zero time. The solution was mixed and the absorbance at 470 nm was recorded for 3 min. The enzyme solutions were diluted sufficiently not to exceed a scΔA of 0.4 per min. A unit of peroxidase was defined as the amount of enzyme that increased the absorbance by 0.1 unit per min at 25 °C.

IAA Oxidase Assay

The oxidation of IAA was usually determined by measuring the residual IAA in reaction mixtures using Salkowski reagent (11, 12). The assay solutions consisted of 0.2 mL of 0.1 M sodium acetate (pH 4.5), 0.3 mL of 1 mM Mn^{2+}, 0.2 mL of 1 mM DCP, 0.4 mL of water, 0.3 mL of 1 mM IAA, and 0.1 ml of enzyme solution. After 5.5 min at 30 °C, 2.0 mL of Salkowski reagent (15 mL of 0.5 M $FeCl_3$, 500 mL of water, and 300 mL of concentrated sulfuric acid) were added. The A at 525 nm was determined after the samples were stored for 30 min in the dark. Absorbances were converted to μmols of residual IAA using a standard curve for IAA. Some samples were assayed for IAA oxidation by measuring the increase in A at 247 nm (24, 30).

RESULTS

Oxidation of IAA by Tomato Peroxidase

Preliminary studies indicated that tomato anionic peroxidase was capable of oxidizing IAA but high concentrations of the enzyme were required. In the standard reaction mixture buffered with 13 mM acetate (pH 4.5), 0.2 μmol of IAA was oxidized in 5 min by 650 nM tomato peroxidase. The reaction rate remained low when acetate was replaced with 13 mM succinate or citrate and decreased as the pH was raised to 6.0 for all three buffers. In contrast, the same rate of IAA oxidation in acetate buffer (pH 4.5), required only 0.9 nM horseradish cationic peroxidase. These observations are consistent with the report by Kokkinakis and Brooks (15) that tomato peroxidase was much less effective than horseradish peroxidase in the oxidation of IAA. But as other buffers were evaluated, the oxidation of IAA by tomato peroxidase was found to be greatly enhanced by some anions.

Phosphate has often been used as the buffer for studying IAA oxidation by peroxidases (11, 12, 30, 33). The addition of low concentrations of the anion adjusted to pH 4.5 to reaction mixtures buffered by 13 mM acetate (pH 4.5), had no effect on oxidation of IAA by tomato peroxidase. As the phosphate concentration exceeded a minimal or threshold level of about 2 mM, the rate of oxidation increased sharply (Fig. 1). Activation was maximal at about 100 mM phosphate and concentrations as high as 330 mM were only slightly inhibitory. The activation by phosphate allowed the use of much less tomato peroxidase in the reaction mixture for a measurable rate of oxidation. In the presence of 100 mM phosphate, only 0.45 nM peroxidase oxidized 0.2 μmol of IAA in 5 min. A similar rate of oxidation required 650 nM peroxidase in the absence of phosphate, as indicated above. Phosphate derivatives including glucose-1-phosphate, 2-glycerophosphate, and phytic acid activated tomato peroxidase but

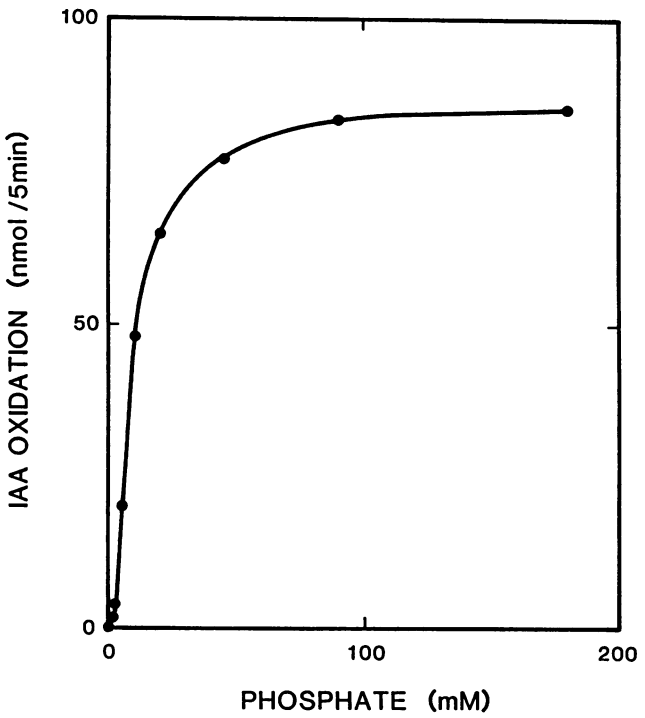

Figure 1. Effect of phosphate concentration on IAA oxidation by tomato peroxidase.

less than free phosphate. Arsenate was somewhat less effective than phosphate and a comparably high concentration (110 mM) was required for maximal activation.

Pyrophosphate activated the peroxidase to the same extent as phosphate but at much lower concentrations (Table I). Other anions containing the pyrophosphate group such as cocarboxylase, ADP and UDP activated the enzyme but less effectively than pyrophosphate.

Of the relatively large number of anions tested, NTA was the most effective activator of tomato peroxidase. This chelator was better than phosphate not only in terms of the amount of peroxidase to obtain an oxidation rate of 0.2 μmol IAA/5 min (Table I) but also much lower concentrations of NTA were required (Fig. 2). Activation of the reaction was appreciable at a NTA concentration of only 1 μM and optimal at about 60 μM. Higher concentrations of NTA inhibited the oxidation of IAA, with complete inhibition by 2.5 mM NTA. The analogue nitrilotripropionate was much less effective than NTA, with a small amount of activation of the oxidation at a concentration of 3 mM. Another chelator, EGTA, also was less effective than NTA (Table I), whereas EDTA, 1,2-cyclohexanediamine tetraacetic acid, pentanedione, and hydroxyquinoline neither activated nor inhibited the reaction.

Oxalate was also a better activator of tomato peroxidase than was phosphate (Table I). Like NTA, oxalate was effective at relatively low concentrations (Fig. 3). But unlike NTA and some other activators such as pyrophosphate, activation by oxalate occurred slowly with increasing concentrations, suggesting a requirement for a threshold level as for phosphate. Optimal activation was obtained with 210 μM oxalate. Increasing concentration of oxalate resulted in much sharper inhibition of IAA oxidation than observed for NTA. Oxamic acid, the monoamide of oxalic acid, had no effect on the reaction.

Malonate, the next higher homolog of the aliphatic dicarboxylate series, was about half as effective as oxalate at an optimal concentration of 1.8 mM (Table I). Ketomalonate was a weaker activator of peroxidase but its optimal concentration was lower than that for oxalate and malonate. This anion changed from an activator to an inhibitor at relatively low concentrations, as shown in (Fig. 4). The inhibition by high concentrations of ketomalonate was not reversed by adding oxalate or other activators to the reaction mixture.

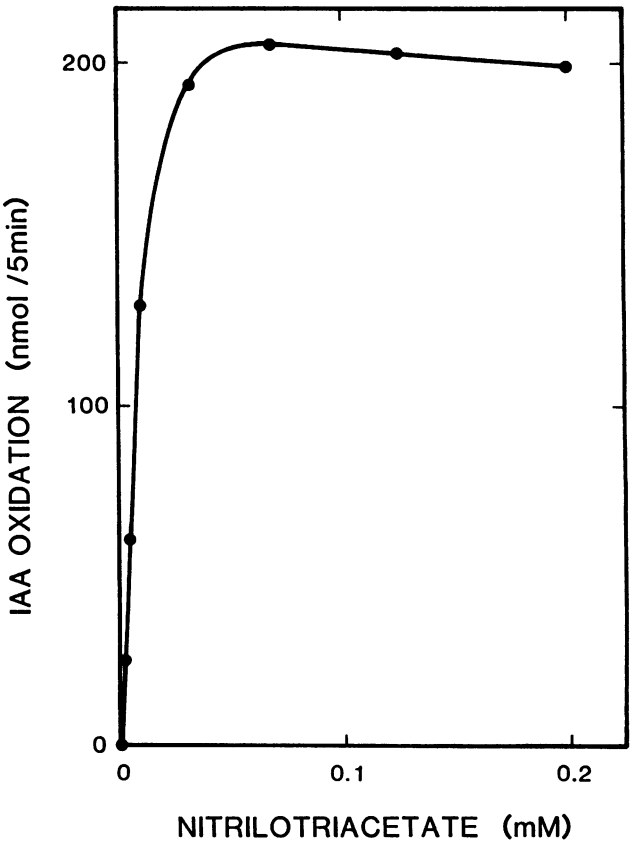

Figure 2. Effect of NTA concentration on IAA oxidation by tomato peroxidase.

Succinate was one of the more effective activators of peroxidase but only at very high concentrations (Table I). The optimum concentration for succinate was about 300 mM, and even 1 M succinate was not inhibitory. Maleate and glutarate were similar to succinate in effectiveness and optimal concentrations, but fumarate and adipate were much less effective. Malate and tartrate, hydroxy derivatives of succinate, were less effective than succinate but with much lower optimal concentrations (Table I). Citrate was a good activator at 0.15 mM, but it became inhibitory of IAA oxidation at relatively low concentrations, much like ketomalonate.

The monocarboxylate homologs formate, acetate and propionate, were without effect on IAA oxidation at concentrations as high as 330 mM. The hydroxy analogs glycolate and lactate were very weak activators whereas the keto derivatives glyoxylate and pyruvate were moderately effective (Table I). A variety of amino acids including glycine, arginine, aspartate, and hydroxyproline had no effects on IAA oxidation.

Effects of pH, Mn^{2+} and DCP. The effect of pH on IAA oxidation by tomato peroxidase was determined for three of the best activators, phosphate, oxalate and NTA. Buffers were prepared by adding each anion to 13 mM acetate-Mes buffers and adjusting the pH with HCl or NaOH. A rather sharp

Table I. *Effects of Anions on Oxidation of IAA by Tomato Peroxidase*

The standard reaction mixture contained 13 mM acetate (pH 4.5) and 0.17 nM tomato peroxidase.

Anion	Optimum Concentration	IAA Oxidation
	mM	*nmol/5 min*
Phosphate	105	84
Pyrophosphate	0.18	79
NTA	0.06	210
EGTA	2.0	52
Oxalate	0.21	146
Malonate	1.7	45
Ketomalonate	0.06	32
Succinate	300	165
Malate	2.6	34
Tartrate	16.0	16
Citrate	0.15	26
Glyoxylate	3.3	17
Pyruvate	20.0	12

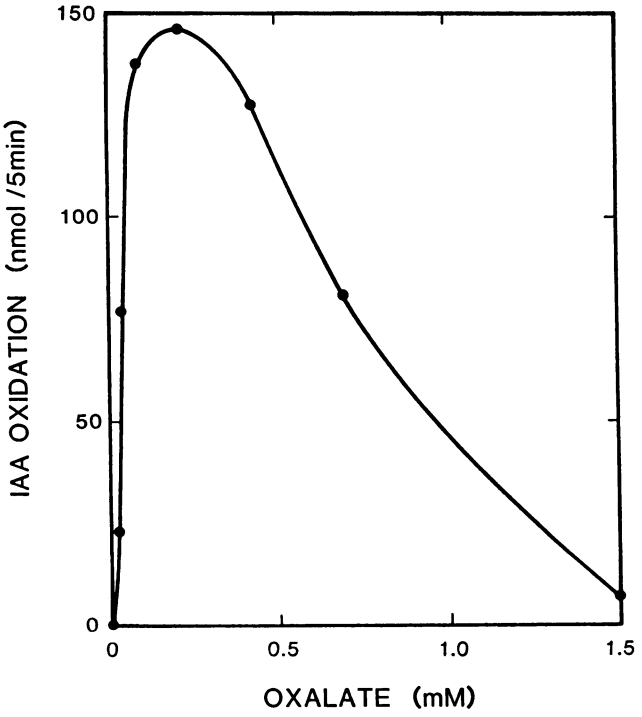

Figure 3. Effect of oxalate concentration on IAA oxidation by tomato peroxidase.

optimum was obtained at pH 4.5 in the presence of 100 mM phosphate (Fig. 5). Oxalate and especially NTA yielded broader activity curves in relation to pH with maxima near pH 4.5.

DCP promoted IAA oxidation by tomato peroxidase, as reported by others (11, 12, 21). At optimal concentrations of anions and Mn^{2+} in the presence of low levels of peroxidase (0.2 nM), the rate of oxidation increased with DCP concentration to a maximum at 0.13 mM DCP. But this monophenol was not required for IAA oxidation at higher levels of peroxidase. Thus, an oxidation rate of 0.2 μmol IAA/5 min was obtained in the absence of DCP when the concentration of peroxidase was increased to 2 nM. The reaction occurred optimally at pH 3.5 in the absence of DCP. The enzyme responded to anions the same way as in the presence of DCP, with activation at low concentrations and inhibition at higher concentrations (data not shown).

In contrast to the effects of anions and monophenols, tomato peroxidase exhibited an absolute requirement for Mn^{2+}. This cation could not be replaced by Co^{2+}, Zn^{2+}, Fe^{2+}, Cr^{2+}, or others, and the enzyme was completely inactive on IAA in its absence. At optimal concentrations of various anions, oxidation of IAA increased with Mn^{2+} concentration to a maximum at 0.2 mM Mn^{2+}. Higher concentrations of Mn^{2+} were inhibitory, especially as the optimal levels of anions were exceeded.

Oxidation of IAA by Horseradish Peroxidases

As indicated above, the cationic peroxidase from horseradish was an effective catalyst of IAA oxidation even in acetate buffer. This enzyme also was activated by anions but the effects were not as dramatic as for tomato peroxidase. At an optimal concentration of 0.24 mM oxalate, the concentration of cationic peroxidase required to oxidize 0.2 μmol of IAA/5 min was reduced to 0.12 nM from 0.9 nM in acetate buffer. The effectiveness of the various anions on this enzyme differed somewhat from that for tomato peroxidase (Table II).

The anionic peroxidase from horseradish was much less effective than the cationic isoenzyme in the oxidation of IAA in acetate buffer. At pH 4.5, 290 nM anionic peroxidase oxidized 0.2 μmol IAA/5 min. The addition of 50 μM NTA to the reaction mixture decreased the amount of enzyme for the same rate of oxidation to only 0.22 nM. The large effects of anions on this enzyme and their order of effectiveness resembled that for tomato anionic peroxidase (Table II).

Oxidation of IAA by Lactoperoxidase

Reaction mixtures containing 0.5 μg lactoperoxidase and acetate buffer (pH 4.5) oxidized 0.12 μmol IAA/5 min. Oxidation by this enzyme was activated by anions with a maximal increase in activity of about 5-fold. The most effective anion was phosphate, followed by NTA, malate, pyrophosphate, and oxalate.

Oxidation of IAA by other Porphyrin Proteins

Myoglobin (50 μg), hemoglobin (50 μg), and catalase (100 μg) in 1.5 mL of standard reaction mixture containing 0.2 mM oxalate catalyzed the oxidation of 0.2 μmol IAA/5 min.

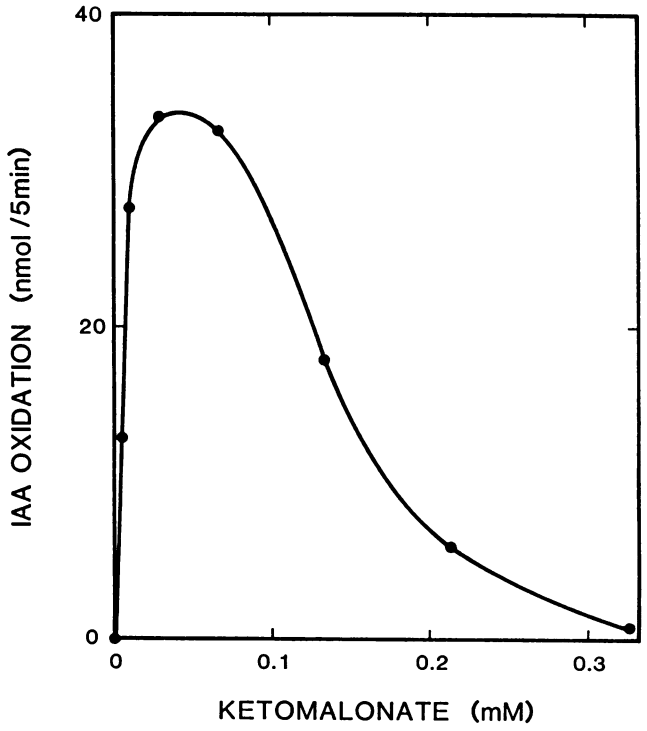

Figure 4. Effect of ketomalonate concentration on IAA oxidation by tomato peroxidase.

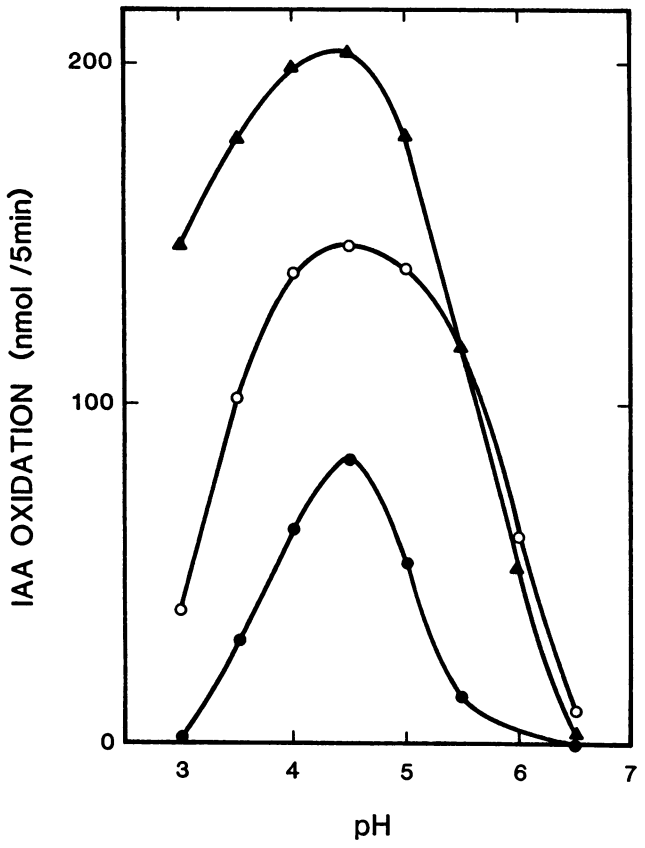

Figure 5. Effect of pH on the oxidation of IAA by tomato peroxidase in the presence of 100 mM phosphate (●), 0.06 mm NTA (▲), and 0.20 mM oxalate (○).

Measurable oxidation did not occur at pH 3 to 6 in the absence of oxalate or other anions such as phosphate and NTA. Except for the much higher levels of porphyrin proteins, the reactions resembled that for tomato peroxidase. In contrast, Cyt *c* did not catalyze IAA oxidation over broad ranges of protein and oxalate or NTA concentrations at pH 3 to 6. But the microperoxidases MP-9 and MP-11 prepared by hydrolysis of Cyt *c* (6) functioned as IAA oxidases. Concentrations of these porphyrin peptides in the range of 1 μM were required. Measurable oxidation was obtained only in the presence of anions such as oxalate (Table II). The microperoxidases were optimally active at pH 3.5.

Inhibition of IAA Oxidation

The IAA oxidase activities of tomato and horseradish peroxidases were strongly inhibited by low concentrations of cyanide, azide, and hydroxylamine (Table III), well-known inhibitors of enzymes that contain porphyrin. Various aldehyde reagents such as dinitrophenylhydrazine, aminooxyacetate, and DOPA were also strong inhibitors while L-α-(2-aminoethoxyvinyl)-glycine had no effect. The peroxidases were inhibited by Cu^{2+}, and except for the cationic horseradish peroxidase, by diethyldithiocarbamate which is usually considered to be a copper-chelator. Tiron and diphenols such as catechol were very effective inhibitors. The response of microperoxidase to the inhibitors was very similar to that for peroxidases except for a higher tolerance to cyanide, with complete inhibition by 1.5 mM cyanide.

DISCUSSION

It has long been known that IAA in plant tissues is lost during the preparation of aqueous extracts. The rapid disappearance of the hormone is due to oxidation by peroxidase (18, 19). The reaction requires O_2 and Mn^{2+} and is strongly promoted by monophenols present in plant tissues. The present study shows that the oxidation of IAA by peroxidases is greatly influenced by various anions including several that are common in plants. A possible reason why the activation by anions had not been recognized previously is that phosphate, itself one of the best activators, has generally been used as the buffer in studying IAA oxidation. Some anions promote the reaction as well as phosphate but at much lower concentrations. For example, the optimal concentration of phosphate was about 100 mM but only 50 μM for NTA (Table I). The anions exert maximal activation over a relatively narrow concentration range and inhibit the oxidation at higher concentrations and this may further explain why this phenomenon has been overlooked.

Except for phosphate and pyrophosphate, the best activators of IAA oxidation were di- and tricarboxylates. But the relationship between anion effectiveness and factors such as acidic ionization and intercharge distance is not obvious, suggesting that a suitable combination of properties is required. The effective anions are all known to be metal chelators although some classical chelators like EDTA and pentanedione were without effect. Presumably activation of the oxidation by anions is due to interaction with Mn^{2+} to yield a more reactive complex of the metal ion.

The ability of peroxidase to oxidize IAA is clearly a function

Table II. *Effects of Anions on IAA Oxidation by Microperoxidase and Horseradish Peroxidases*

The reaction mixtures contained 0.22 nM type VIII peroxidase; 0.12 nM type IX peroxidase, and 0.15 μM microperoxidase MP-11, respectively. The horseradish peroxidases were assayed at pH 4.5 and microperoxidase was assayed at pH 3.5.

Anion	Anion Concentration	Peroxidase		
		Type VIII	Type IX	MP-11
	mM	*nmol/5 min*		
Phosphate	105	140	168	212
Pyrophosphate	0.18	66	62	33
NTA	0.06	205	112	115
EGTA	2.0	64	39	38
Oxalate	0.21	114	220	94
Malonate	1.7	83	61	42
Ketomalonate	0.06	108	30	26
Malonate	2.6	90	62	77
Citrate	0.15	47	24	38

Table III. *Effects of Inhibitors on Oxidation of IAA by Peroxidases*

Inhibitors were added to standard reaction mixtures containing 0.21 mM oxalate. Concentrations of peroxidases were 0.17 nM tomato, 0.22 nM horseradish type VIII, 0.12 mM horseradish type IX, and 0.15 μM MP-11.

Inhibitor	Inhibitor Concentration	Peroxidase			
		Tomato	Type VIII	Type IX	MP-11
	μM	% inhibition			
Cu^{2+}	15	40	72	100	100
Cyanide	15	100	100	77	0
Azide	150	100	25	56	80
Hydroxylamine	10	100	100	22	25
DNP[a]	10	100	33	20	100
Aminooxyacetate	100	40	100	29	71
DOPA[b]	10	100	100	53	100
Catechol	10	100	100	35	100
DEDTC[c]	50	44	100	0	40
Tiron	100	100	100	100	100

[a] Dinitrophenylhydrazine. [b] Dihydroxyphenylalanine. [c] Diethyldithiocarbamate.

of the prosthetic group porphyrin. I did not detect IAA oxidase activity for apoperoxidase, contrary to the report by Siegel and Galston (26). Microperoxidase, a hydrolytic product of Cyt *c* with only 11 amino acids attached to iron-prophyrin, was a relatively effective IAA oxidase in the presence of appropriate cofactors. Similarly, myoglobin, hemoglobin, and catalase oxidized IAA appreciably at conditions optimized for tomato peroxidase. These hematoporphyrin catalysts were much less effective than the three peroxidases studied, and Cyt *c* appeared to be incapable of oxidizing IAA. These observations suggest that while the porphyrin group is essential for IAA oxidation, the overall performance of the catalyst is determined by the apoprotein. The great efficiency of peroxidase indicates that the apoprotein enhances the reactivity of the hematoporphyrin. In contrast, the lack of oxidation with Cyt *c* must be due to interference from the peptide because removal of most of it to produce microperoxidase yields an active IAA oxidase.

Peroxidase is widely distributed in higher plants but the physiological role of this enzyme remains elusive. It has been implicated in lignification, ethylene biosynthesis, proline hydroxylation, IAA degradation, and other functions (10). The oxidation of IAA has received considerable attention because of the potential role of peroxidase in regulating IAA-mediated growth. The *in vitro* reaction catalyzed by horseradish peroxidase involves decarboxylation of IAA to yield 3-methyleneoxindole and other products (18). Although up to ten products have been identified in seedlings supplied radiolabeled IAA, the most prominent was oxindoleacetic acid (17, 22). Oxindoleacetic acid has also been identified as an endogenous component of maize (23). Reinecke and Bandurski (22, 23) concluded from this evidence that most of the IAA is catabolyzed by a mechanism other than decarboxylation by peroxidase.

The results of the present study suggest that peroxidase is not involved in IAA destruction in intact plants. Oxidation of IAA by other hematoporphrins indicates that the reaction may be catalyzed by free radicals generated by the porphrin groups of the proteins in the presence of appropriate cofactors. The oxidation by peroxidase is an extremely effective reaction but is optimally expressed in the presence of a relatively large number of cofactors, *i.e.* Mn^{2+}, monophenol, dicarboxylic acid, and O_2. For this reason, I suggest that oxidation of IAA by peroxidase may be important under circumstances where all of these components mix freely as during injury to plants. Disruption of membranes would allow vacuolar contents to interact with peroxidase in cell walls. A possible consequence would be that IAA present in the injured zone would be rapidly destroyed by the activated peroxidase. The depletion of IAA could serve as a signal to intact neighboring cells to shift from normal functions to defensive and healing processes.

Among the proposed functions for IAA are that it inhibits ripening (7–9) and prevents abscission of fruits (28). Haard (10) presented evidence that IAA declines concomitantly with ripening of tomatoes. A common response of unripe fruit tissue to injury by insects or other factors is premature ripening around the damaged area. The destruction of IAA in the damaged cells by peroxidase activated by components released by the injury could initiate ripening in the adjacent cells.

There has been considerable interest recently in the stimulation of ethylene production in tomatoes and other fruits by infiltration of enzymes (2, 3) or carbohydrate fragments from cell walls (4, 31). Some polysaccharide fragments appear to regulate various plant functions besides ethylene production and have been named oligosaccharins (1). Preliminary studies with an enzymatic hydrolyzate of polygalacturonic acid (Sigma Chemical Co.) indicated that the mixture of pectic fragments activated IAA oxidation by peroxidase. Separation and evaluation of the individual components of the hydrolyzate revealed that normal oligogalacturonides were not responsible for the activation. The effective components were oxidized oligogalacturonides, with single residues of galactaric acid. The results suggest that oligosaccharin activity attributed to oligogalactuonides may be due to oxidized analogs. A

possible mode of action is an activation of IAA destruction by peroxidase leading to reduced levels of IAA and concomitant physiological changes. Details of the study on oligogalacturonides will be published separately.

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