# Peroxidase-catalyzed IAA-oxidation in presence of cofactors and auxin protectors isolated from *Eriophyes* incited *Zizyphus* gall tissue

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(*Received* : 6*th* Nov. 1984)

## ABSTRACT

Auxin protectors isolated from *Zizyphus* gall tissue prevent the horse radish peroxidase-catalyzed indole-3-acetic acid oxidation. The possible mechanism of auxin protector induced lag in indole-3-acetic acid oxidation and the influence of cofactors is described here. In absence of auxin protectors, the rate of the oxidation was directly proportional to the enzyme concentration. The preincubation of auxin protectors with manganese decreased the lag period. At lower concentration of 2, 4-dichlorophenol, the indole-3-acetic acid oxidation was enhanced and protector induced lag was reduced. However, higher concentrations were inhibitory. Both hydrogen peroxide and riboflavin abolished the auxin protector induced lag.

## Introduction

A large number of insects and mites incite the tissue of the host plant to initiate galls. These galls may be simple, involving single organ or may be compound where two or more plant organs are concerned in the production of the gall. The insect galls are considered to be highly specialized overgrowths that represent good examples of dependent differentiation. In contrast to non-self-limiting plant galls with etiological factors like bacteria viruses, and genetic constitution, the insect and mite-incited galls are reported to be self-limiting (Braun, 1969). One feature, that is common to all types of plant galls is their abnormal growth hormone metabolism (Stonier 1972 ; Butcher, 1973 ; Arya *et al.*, 1975 ; Tan-

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don and Arya, 1980a, 1982 ; Weiler and Spanier, 1981 ; Amasino and Miller, 1982). *Eriophyes cernuus* incited galls on *Zizyphus jujuba* are of non-self-limiting type, possess tumefacient properties (Tandon *et al.* 1976) and show hyperauxinity and auxin autotrophy (Tandon and Arya, 1980a).

There are many reports regarding the gall tissue as showing low indole-3-acetic acid (IAA) oxidase activity compared to the normal, resulting in hyperauxinity of the former (ref. Tandon and Arya, 1982). On the other hand IAA-oxidase inhibitors have also been reported from gall tissues. The naturally occurring IAA-oxidase inhibitors of phenolic nature range from free low molecular weight compounds such as chlorogenic, ferulic and protoctechuic acids to high molecular weight auxin protectors (Schneider and Wightman, 1974). Auxin protectors have been isolated from crown-gall on sunflower stem (Stonier, 1969 ; Atsumi and Hayashi, 1978), *Synchytrium* incited galls in potato (Haard, 1978) and *Eriophyes* incited *Zizyphus* galls (Tandon and Arya, 1980b). The unregulated synthesis of auxin protectors is considered to be responsible for auxin-autotrophy and hyperauxinity in gall tissues. The auxin protectors are O-dihydroxyphenols (Stonier, 1972 ; Tandon and Arya, 1980 b and c) which prevent IAA-destruction by masking IAA-oxidase (Stonier et al., 1979). An association of auxin protectors, peroxidase, IAA-oxidase and polyphenol oxidase has been established in *Zizyphus* gall tissue (Tandon and Arya, 1982). The present paper deals with the mechanism of auxin protector action on IAA-oxidation as influenced by different cofactors.

## MATERIALS AND METHODS

### Separation of Auxin Protectors

The auxin protectors from *Eriophyes cernuus* Massee incited *Zizyphus jujuba* Lamk. gall tissue were separated by using Sephadex G-200, G-50 and G-15 as described earlier (Tandon and Arya, 1980c). Three auxin protectors, Pr-I (mol. wt. exceeding $2 \times 10^5$ daltons), Pr-II (mol. wt. ca $10^4$ daltons) and Pr-III (mol. wt. ca $2 \times 10^3$ daltons) were isolated.

### Basic Assay for Protector Activity

The reaction mixture used to assay auxin protectors consisted of a mixture of 2, 4-dichlorophenol (DCP), manganese chloride and IAA, each at a final concentration of 0.1 mM and horse radish peroxidase (HRP) at a final concentration of 0.5 $\mu$g, 0.2 $\mu$g, and 0.1 $\mu$g per ml for Pr-I, P-II and Pr-III, respectively. The final volume of the reaction mixture was 10 ml ; all reactions were buffered at pH 6.1 with 20mM potassium phosphate. The reaction mixture was shaken in 25×200 mm test tubes in a shaker at 30° C. To prevent IAA destruction, 0.05 ml of auxin protector was added. 0.5 ml aliquots were drawn from the reaction mixture at various time intervals and mixed with 2.0 ml of Salkowski reagent. The absorbance of the reaction was measured at 530 nm after one hour (Gordon and Weber 1951).

### Enzyme Concentration

Different HRP concentrations (final concentrations 0,25 to 1.0 $\mu$g/ml) were used to study the lag in IAA-oxidation induced by the gall tissue extract. HRP final concentrations ranging from 0.25 to 1.0 $\mu$g/ml for Pr-I, and 0.05 to 0.20 $\mu$g/ml for Pr-II and Pr-III, were used to study the lag in IAA-oxidation.

### Manganese

Effect of manganese on HRP-catalyzed IAA-oxidation in presence and absence of auxin protectors was studied. The auxin protectors were preincubated with $MnCl_2$ in air and in nitrogen separately for 120 min and then auxin protector induced lag was studied.

### Dichlorophenol, hydrogen peroxide and riboflavin

The effects of DCP (0.025 to 0.4 mM), $H_2O_2$ (0.01 mM) and riboflavin (0.2 mM in the light and dark) were studied on HRP-catalyzed IAA-oxidation and on auxin protector induced lag.

## RESULTS

### Effects of different concentrations of HRP

The results presented in Figs. 1, 2, 3 and 4 show that the rate of IAA-oxidation is directly proportional to the concentration of HRP in the absence of the gall tissue extract or auxin protectors. However, in their presence, there was a corresponding decrease or increase in the lag period.

### Effect of Manganese

The IAA-oxidation was slow in the absence of $Mn^{2+}$ when compared to the reaction mixture containing it. Addition of $Mn^{2+}$ reduced the auxin protector induced lag (Figs. 5, 6 and 7). The table-1 shows that auxin protector induced lag was greatly reduced when auxin protectors were preincubated with $Mn^{2+}$ in air and in nitrogen as compared to their preincubations in absence of $Mn^{2+}$.

### Effect of DCP, $H_2O_2$, andRiboflaven

At 0.05 mM DCP (1 mole of DCP per 2 mole of IAA), maximum destruction occurred in the absence of auxin protectors. The auxin protectors induced lags were also lowest at this concentration of DCP (Table 2). Addition of 0.01 mM $H_2O_2$ in the reaction mixture completely eliminated the auxin protector induced lag. The $H_2O_2$ alone did not oxidize IAA during the incubation period tested (Figs. 8, 9 and 10). Riboflavin (0.2 mM) in light eliminated the auxin protector induced lag. The destruction of IAA by riboflavin in light was increased in the presence of auxin protectors. However in dark no destrucion occurred (Fig. 11).

## DISCUSSION

Auxin protectors are substances that prevent peroxidase-catalyzed oxidation of IAA, rather than changing the rate of oxidation. The shape of the curves in Figs. 2 and 3 show that oxidation of IAA by HRP was delayed by the presence of auxin protectors and once the lag period was over the normal oxidation of IAA proceeded. It seems that auxin protectors are themselves oxidized first, and during this period a lag in IAA oxidation is observed. Thus, the auxin protectors are not enzyme inhibitors per se.

Manganese has been found to be a cofactor for many IAA-oxidase systems (Galston and Hillman, 1961) and the manganese nutrition of cotton altered the levels of IAA-oxidase. The preincubation of pea-homo-genates with $Mn^{2+}$-resulted in an increase in IAA-oxidase activity, probably due to the inactivation of IAA-oxidase inhibitor (Furuya and Galston, 1961). At toxic levels of manganese, IAA-oxidase activity in cotton plants increased and IAA-oxidase inhibitor activity was decreased (Morgan et al., 1966). The manganese status of plant may therefore, affect IAA-oxidase activity directly or via its effects on natural cofactors and inhibitors of the system. In present studies, the preincubation of auxin protectors with manganese in air decreased the lag time in IAA-oxidation. Stonier et al. (1968a, b) reported that auxin protectors interacted directly with manganese and not with IAA, DCP or enzyme when these reagents were preincubated singly with the protector. It appears that $Mn^{2+}$ not only oxidizes auxin protectors but also speeds up transfer of electrons from IAA to the electron acceptor DCP in a HRP-catalyzed reaction. These findings are in line with the work by Morgan et al. (1966) for cotton plant, Furuya and Galston (1961) for pea seedlings and Stonier et al. (1968a) for morning glory protectors. The auxin protectors donate electrons more readily than IAA thus causing delay in IAA-oxidation or replenish electrons lost by IAA in a reversible step leading to its oxidation (Stonier et al., 1968b). The factors that speeded the transfer of electrons during the process of IAA-oxidation caused a decrease in the lag period prior to oxidation. As soon as the auxin protectors were depleted of their electrons, they were rendered inactive, and then normal IAA-oxidation proceeded. On the other hand, any substance which could slow the flow of electrons might result in decreased rate of electron transfer from auxin protectors. As a consequence, a lag period would be observed. In present studies, the decrease in HRP concentration resulted in enhancement of the lag period (Figs. 1, 2 and 3). With the increase in molecular weight of auxin protectors their activity enhanced.

The role of $H_2O_2$ in peroxidase catalyzed degradation of IAA has been extensively studied (Grambow, 1982, Grambow and Langenbeck-Schwich, 1983). The auxin protectors were rendered inactive, in present investigations, upon incubation with low concentration of $H_2O_2$ which otherwise had no effect on the rate of HRP-catalyzed oxidation of IAA in absence of auxin protectors. Similarly auxin protector inactivation was also obtained by incubating them with riboflavin in light but not in dark.

The involvement of phenolic compounds in IAA oxidation has been studied in detail (Lee, 1977 ; Lee et al., 1982). The activities of peroxidase and IAA-oxidase can be altered by phenols (both free and bound) and many naturally occurring compounds (Schneider and Wightman, 1974). While monophenols are known to promote IAA-oxidation, the diphenols and polyhydroxyphenols inhibited it. In present studies, at low concentration of DCP, the IAA-oxidation was promoted and the lag period induced by auxin protectors was decreased. However, the IAA-oxidation was inhibited at higher concentrations. Scopoletin, a naturally occurring coumarin, was shown to inhibit sweet potato IAA-oxidase at higher concentrations, whereas lower concentrations of it were stimulatory (Imbert and Wilson, 1970). Sabater et al. (1983) reported that DCP, although a very efficient cofactor, promoted inhibition of oxidation of IAA after a few minutes when the enzyme/substrate ratio was high, presumably through the formulation of a phenol-derivative inhibitor. The inhibitory effect was eliminated by a low concentration of the enzyme. It was further reported that though $Mn^{2+}$ was a weak inhibitor, it synergized the catalytic effect of DCP, perhaps by preventing the formation of inhibitor.

It is quite clear that indole compounds are oxidised by peroxidase and this system is regulated by phenolic compounds. The latter, in turn, are regulated by polyphenol oxidase which is able to convert monophenols (electron acceptors) to O-diphenols (electrons donors) to O-quinones (strong electron acceptors). An increase in polyphenol oxidase activity was reported in *Zizyphus* gall development (Tandon and Arya, 1982). To conclude, it may be pointed out that high concentrations of auxin protectors in *Zizyphus* gall tissue favour the high electronegativity, and prevent peroxidase catalyzed oxidations required for differentiation (Kevers et al., 1984). Auxin protectors, thus are associated with hyperauxinity and abnormal growth resulting in gall formation.

TABLE 1

Effect of Manganese on auxin protector induced lag in IAA-oxidation.

| Preincubation mixture (Duration—120 min) | Duration of lag period (min) | | |
|---|---|---|---|
| | No preincubation | Preincubation in | |
| | | Nitrogen | Air |
| | Pr-I ; 180 | . . . | . . . |
| Pr-I | . . . | 160 | 135 |
| Pr-I + MnCl$_2$ | . . . | 40 | 10 |
| . . . | Pr-II ; 120 | . . . | . . . |
| Pr-II | | 100 | 85 |
| Pr-II + MnCl$_2$ . | . . . | 30 | 5 |
| | Pr-III ; 80 | . . . | . . . |
| Pr-III | . . . | 55 | 30 |
| Pr-III + MnCl$_2$ | . | 15 | 3 |

TABLE 2

Effect of DCP on IAA-oxidation rate and auxin protector induced lag period

| Concentration of DCP (mM) | Time required for 50% IAA destruction in absence of auxin protectors (min) | Duration of lag period induced by auxin protectors (min) | | |
|---|---|---|---|---|
| | | Pr-I | Pr-II | Pr-III |
| 0.025 | 12 | 55 | 33 | 15 |
| 0.05 | 10 | 28 | 20 | 8 |
| 0.10 | 11 | 30 | 20 | 10 |
| 0.20 | 15 | 65 | 45 | 17 |
| 0.40 | 25 | 120 | 75 | 22 |

## REFERENCES

Amasino, R. M., and C. O. Miller. 1982 : Hormonal control of tobacco crown-gall tumor morphology. *Plant Physiol.* 64 : 389-392.

Arya, H. C., G. S. Vyas and P. Tandon. 1975 : The problem of tumor formation in plants, 270-279. *In* H. Y. Mohan Ram, J. J. Shah, and C. K. Shah (eds.), Form, Structure and Function in plants. Sarita Publishers, Meerut, India.

Atsumi, S., and T. Hayashi. 1978 : The relationship between auxin concentration, axuin protection and auxin destruction in crown-gall cells culture *in vitro* Plant and Cell *Physiol.* 19 : 1391-1397.

Braun, A. C. 1969 : Abnormal growth in plants, 379-420. *In* F. C. Steward (ed.), Plant Physiology, a Treatise, Vol. VB. Academic Press, New York.

Butcher, D. N. 1973 : The origins, characteristics and culture of plant tumor cells, 356-391. *In* H. E. Street (ed.), Plant tissue and cell culture, Botanical monographs 11. Blackwell Scientific Publications, Oxford London Edinburgh Melbourne.

Furuya, M., and A. W. Galston. 1961 : Effects of in vitro preincubation with cofactors on the activity of indole acetic acid oxidase of peas. *Physiol. Plantarum*, 14 : 750-766.

Galston, A. W., and W. S. Hillman. 1961 : The degradation of auxin,. 647-670. *In* Hand buch Der Pflanzenphysiologie Vol. 14, Springer-Verlag, Berlin.

Gordon, S. A. and, R. P. Weber. 1951 : Colorimetric estimation of IAA. *Plant. Physiol.* 26 : 192-195.

Grambow, H. J. 1982 : On the role of hydrogen peroxide in peroxidase catalyzed metabolism of indole-3-acetic acid. *Z. Naturforsch* 37 : 884-888.

Grambow, H. J., and B. Langenbect Schwich. 1983 : The relationship between oxidase activity, peroxidase activity, hydrogen peroxide, and phenolic compounds with the degradation of indole-3-acetic acid *in vitro*. *Planta* 157 : 131-137.

Heard, N. F. 1978 : Isolation and partial characterization of auxin protectors from *Synchytrium endobioticum* incited tumors in potato. *Physiological Plant Pathology* 13 : 223-232.

Imbert, M. P., and L. A. Wilson. 1970 : Stimulatory and inhibitory effects of scopoletin on IAA-oxidase preparations from sweet potato. *Phytochem.* 9 : 1787-1794.

Kevers, C., M. Coumans, W. De Greef, M. Jacobs, and T. Gaspar. 1981 : Organogenesis in habituated sugarbeet callus : Auxin content and protectors, peroxidase pattern and inhibitors, *Zeitschrift Für Pflanzenphysiologie* 101 : 79-87.

Lee, T. T. 1977 : Role of phenolic inhibitors in peroxidase mediated degradation of indole-3-acetic acid. *Plant Physiol.* 59 : 372-375.

Lee, T. T., A. N. Starratt, and J. J. Jevnikar 1982 : Regulation of enzymic oxidation of indole-3-acetic acid by phenols : Structure-activity relationships. *Phytochem.* 21 : 517-524.

Morgan, P. W., H. E. Joham, and J. V. Amin. 1966 : Effect of manganese toxicity on the indoleacetic acid oxidase system of cotton. *Plant Physiol.* 41 :718-724.

Sabater, F., J. Sanchez-Brauo and M. Acosta. 1983 : Effects of enzyme/ substrate ratio and of cofactors on the oxidation products of indole-3-acetic acid catalyzed by peroxidase. *Reuista Espanola De Fisiologia* 39 : 169-174.

Schneider, E. A., and F. Wightman. 1974 : Metabolism of auxin in higher plants. *Ann. Rev. Plant Physiol.* 25 : 487-513.

Stonier, T. 1969 : Studies on auxin protectors VII. Association of auxin protectors with crown gall development in sunflower stems. *Plant Physiol.* 44 : 1169-1174.

Stonier, T. 1972 : The role of auxin protectors in autonomous growth, P. 423-435. *In* M. L. Hirth G. Morel (eds.), Les cultures de tissus de plantes. (Proc. Second Intern Conf. on Plant. Tissue Cultures. Strasbourg, France, 1970), Calloq. Internat. C. N. R. S. (France) No. 193.

Stonier, T., F. Rodriguez-Tormes, and Y. Yoneda. 1968a. Studies on auxin protectors IV. The effect of manganese on auxin Protector-I of the Japanese morning glory. *Plant Physiol.* 43 : 69-72.

Stonier, T., Y. Yoneda, and F. Rodriquez-Tormes. 1968b. Studies on auxin protectors V. On the Mechanism of IAA protection by Protectors-I of the Japanese morning glory. *Plant Physiol* 43 : 1141-1145.

Stonier, T., S. Stasinos, and K.B.S. Murthy Reddy. 1979 : The masking of peroxidase-catalyzed oxidation of indole-3-acetic acid in *Vigna*. *Phytochem.* 18 : 25-28.

Tandon, P., G. S. Vyas, and N. C. Arya. 1976 : Mechanism of *in vitro* gall introduction in *Zizyhpus*. *Experientia* 32 : 563-564.

Tandon, P. and H. C. Arya. 1980a. Auxin autotrophy and hyperauxinity of *Zizyphus* gall tissues. *Biochem. Physiol. Pflanzen.* 175 : 537-541.

Tandon, P., and H. C. Arya. 1980b. Presence of auxin protectors in *Eriophyes* induced *Zizyphus* stem galls. *Experientia* 36 : 958-959.

Tandon, P., and H.C. Arya. 1980c : Separation of auxin protectors from *Zizyphus* gall tissue by Sephadex gel filtration. *Curr. Sci.* 49 : 864-865.

Tandon, P., and H. C. Arya. 1982 : Association of auxin protectors. Peroxidase, indoleacetic acid oxidase and polyphenol oxidase in *Zizy phus* gall and normal stem tissues grown in culture. *Biochem. Physiol. Pflanzen.* 177 : 114-124.

Weiler, E. W., and K. Spanier. 1981 : Phytohormones in the formation of crown-gall tumors. *Planta* 153 : 326-337.

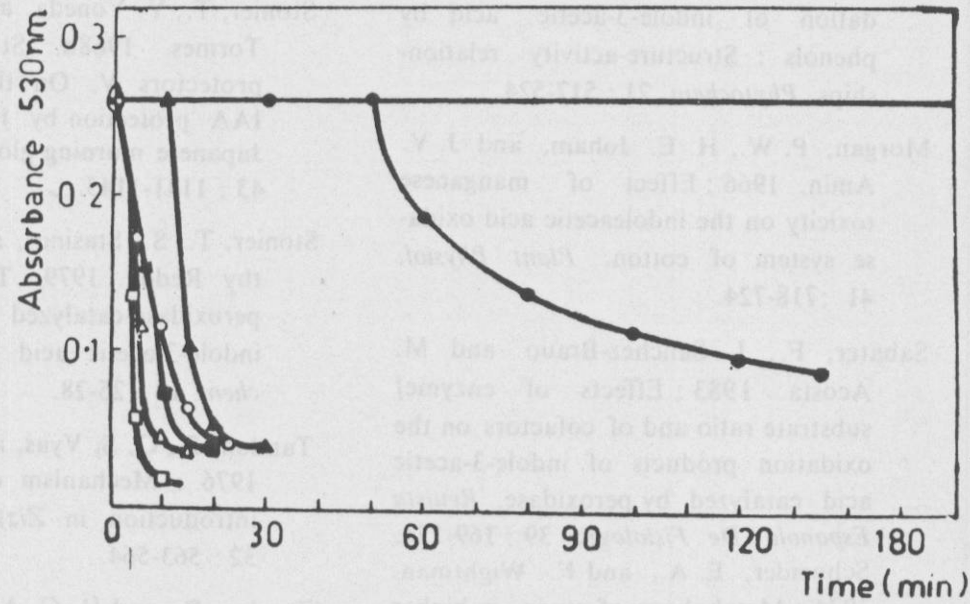

Fig. 1. Effect of HRP concentration on the gall tissue extract induced lag in IAA-oxidation. HRP at a final concentration of 0.25 $\mu$g/ml (0), 0.5 $\mu$g/ml (△) and 1.0 $\mu$g/ml (□). Solid symbols, represent the corresponding enzyme concentration in presence of gall tissue extract (0.1 mg fresh wt. of the tissue).

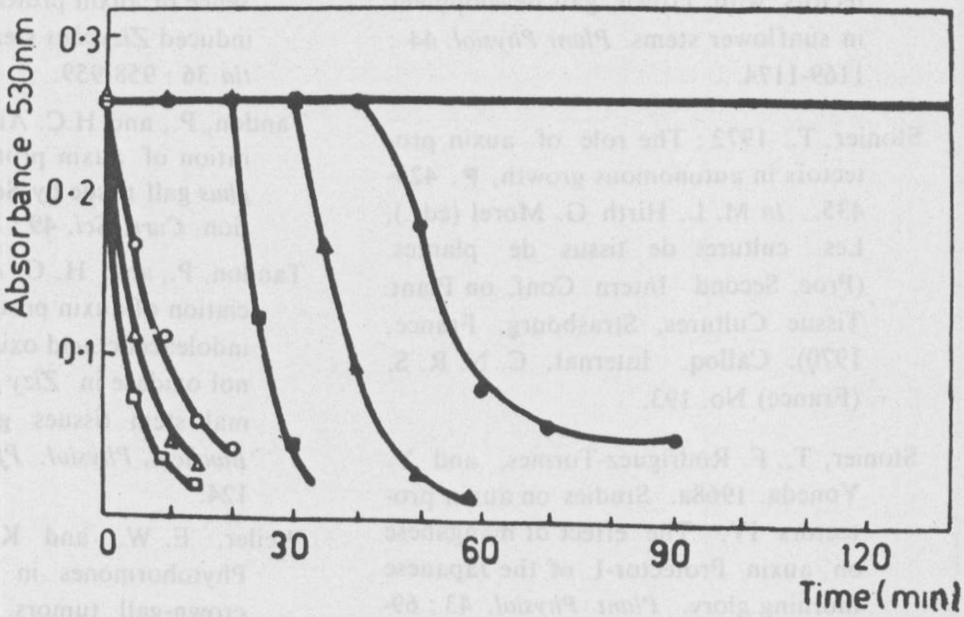

Fig. 2. Effect of HRP concentration on the Pr-I induced lag in IAA-oxidation. HRP at a final concentration of 0.25 $\mu$g/ml (0), 0.5 $\mu$g/ml (△) and 1.0 $\mu$g/ml (□). Solid symbols represent the corresponding enzyme concentration in presence of Pr-I.

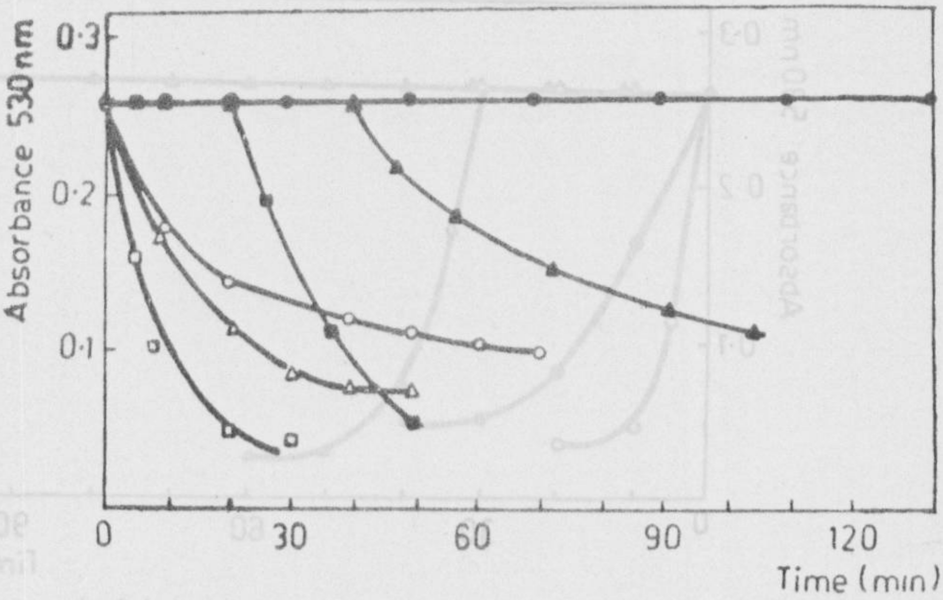

Fig. 3. Effect of HRP concentration on the Pr-II induced lag in IAA-oxidation. HRP at a final concentration of 0.05 μg/ml (0), 0.1 μg/ml (△) and 0.2 μg/ml (□). Solid symbols represent the corresponding enzyme concentration in presence of Pr-II.

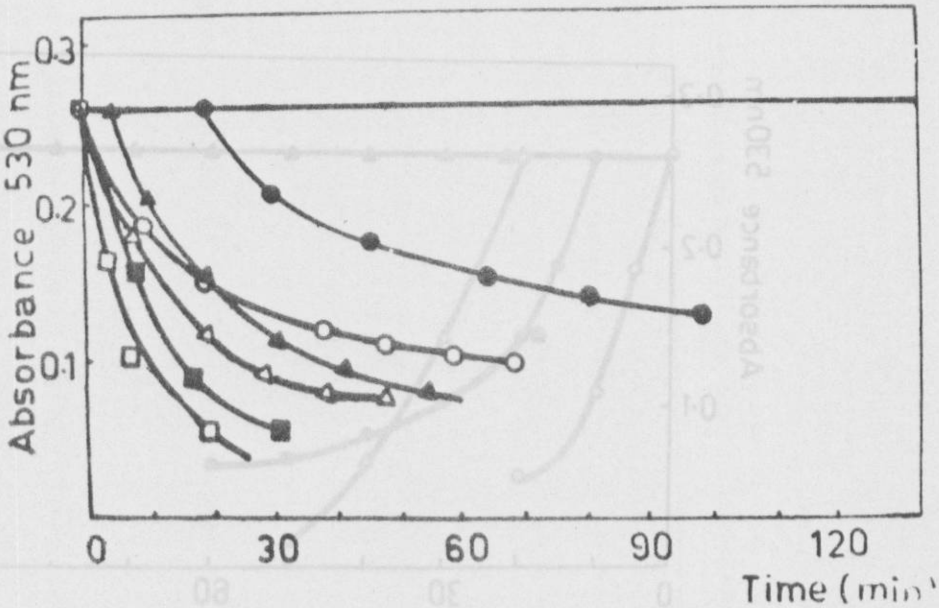

Fig. 4. Effect of HRP concentration on Pr-III induced lag in IAA-oxidation. HRP at a final concentration of 0.05 μg/ml (0), 0.1 μg/ml (△) and 0.2 μg/ml (□). Solid symbols represent the corresponding enzyme concentration in presence of Pr-III.

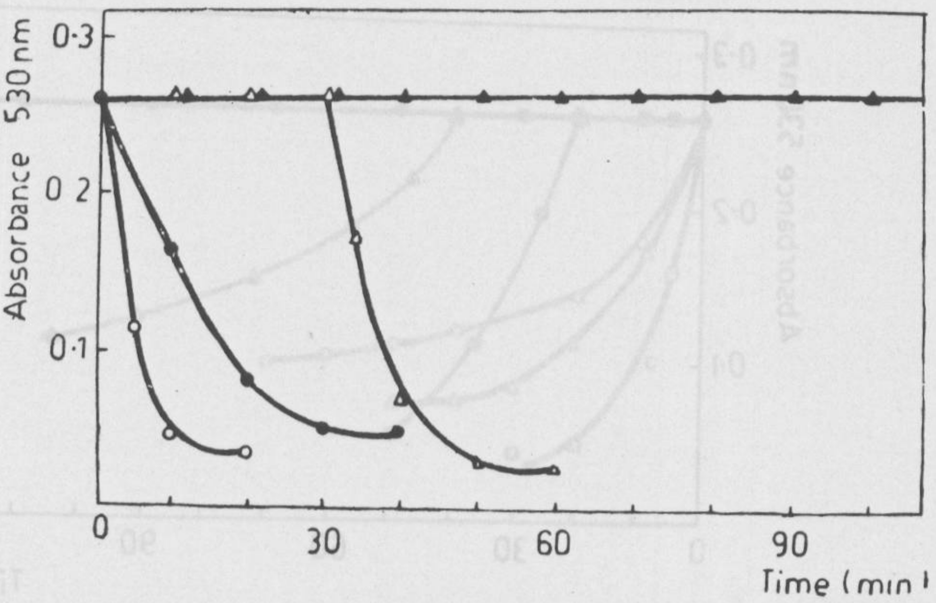

Fig. 5. Effect of manganese on HRP (0.5 $\mu$g/ml final concentration) catalyzed IAA-oxidation in presence and absence of Pr-I. 0 : +Mn,-Pr-I ; 0 : – Mn,-Pr-I ; Δ :+Mn,+Pr-I; Δ : –Mn,+Pr-I.

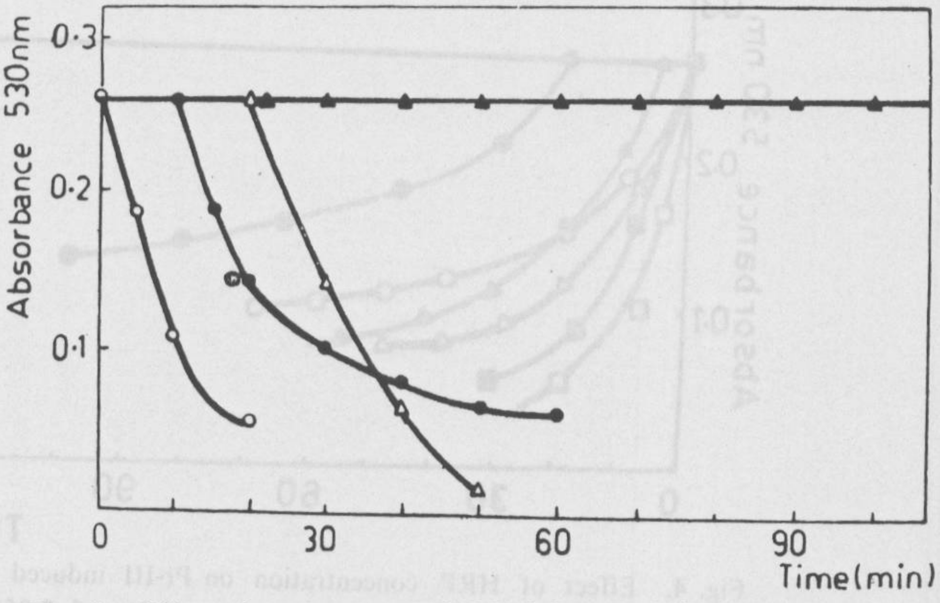

Fig. 6. Effect of manganese on HRP (0.2 $\mu$g/ml final concentration) catalyzed IAA-oxidation in presence and absence of Pr-II. 0 : +Mn,-Pr- II ; ■ : -Mn,-Pr-II ;Δ:+Mn,+Pr-II ;Δ: -Mn,+Pr-II.

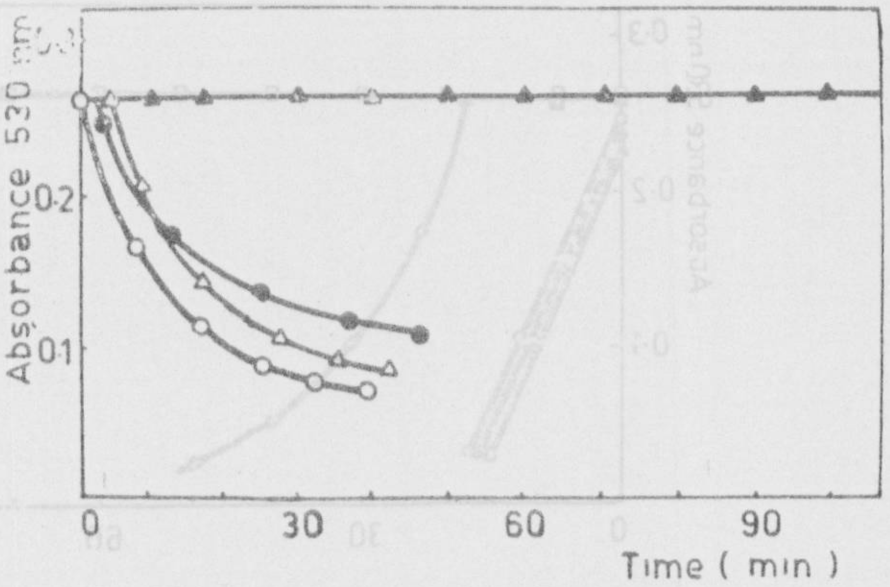

Fig. 7. Effect of Manganese on HRP (0.1 $\mu$g/ml final concentration) catalyzed IAA-oxidation in presence or absence of Pr-III. 0 : +Mn,-Pr-III ; ● : -Mn,-Pr-III; △ : +Mn, + Pr-III ; △ : -Mn, + Pr-III.

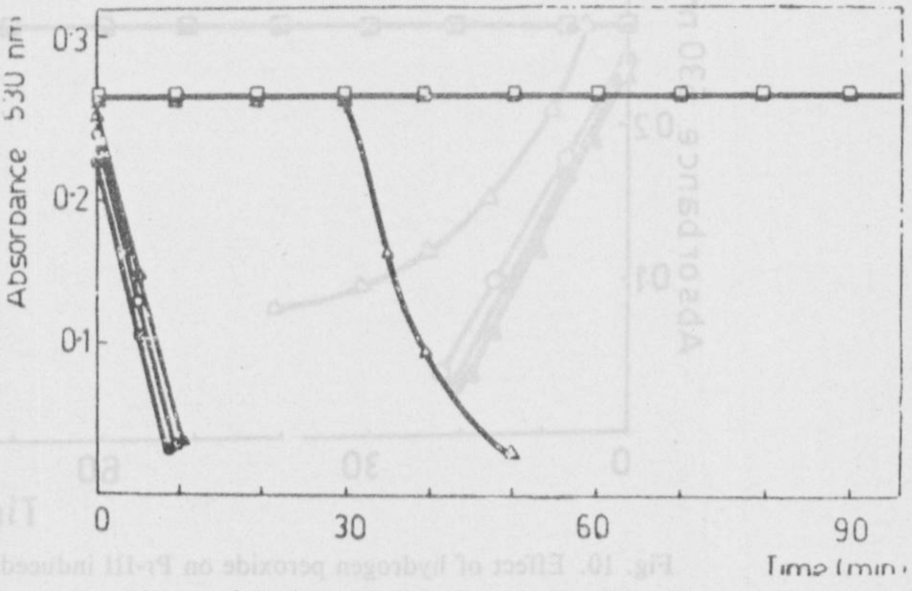

Fig. 8. Effect of hydrogen peroxide on Pr-I induced lag in IAA-oxidation. 0 : HRP only ; ● : HRP + $H_2O_2$ ; △ : HRP + Pr-I ; △ : HRP+Pr-I+$H_2O_2$ ; □ — HRP, -Pr-I ; ■ :-HRP,-Pr-I, +$H_2O_2$.

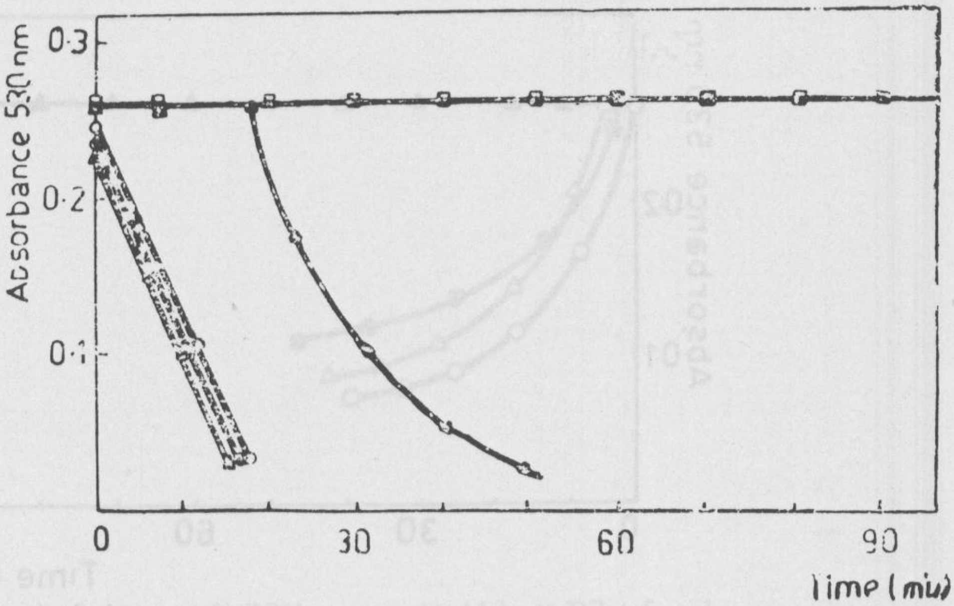

Fig. 9. Effect of hydrogen peroxide on Pr-II induced lag in IAA-oxidation. 0 : HRP only ; ● : HRP+ $H_2O_2$ ; △ : HRP+Pr-II ; △ : HRP+Pr-II+$H_2O_2$ ; ☐ : -HRP, -Pr-II; ■ :-HRP,-Pr-II, +$H_2O_2$.

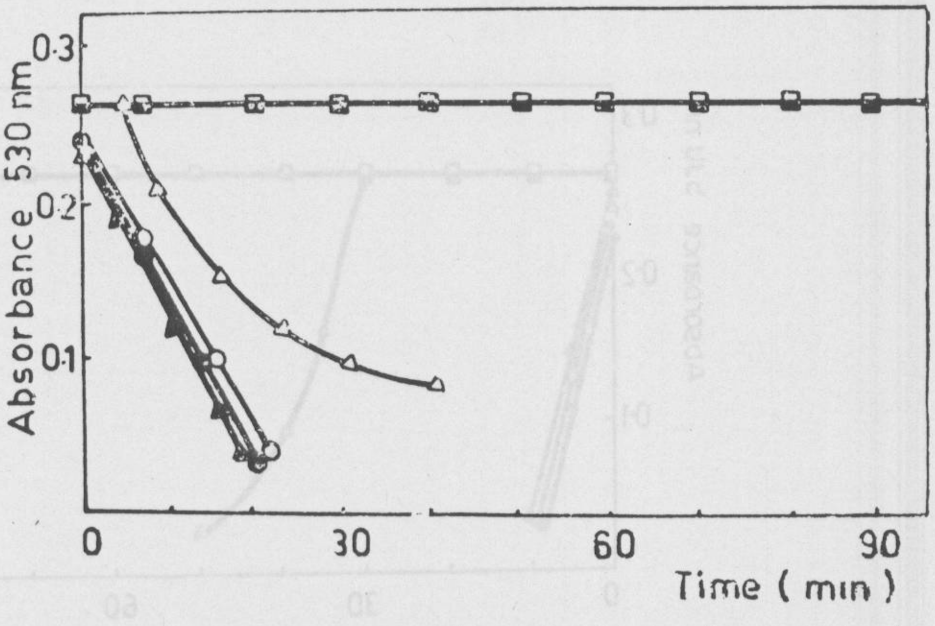

Fig. 10. Effect of hydrogen peroxide on Pr-III induced lag in IAA-oxidation. 0 : HRP only ; ● : HRP+$H_2O_2$ ; △ : HRP+Pr-III ; △ : HRP+Pr-III+$H_2O_2$ ; ☐ : -HRP, -Pr-III ; ■ : -HRP, -Pr-III, +$H_2O_2$.

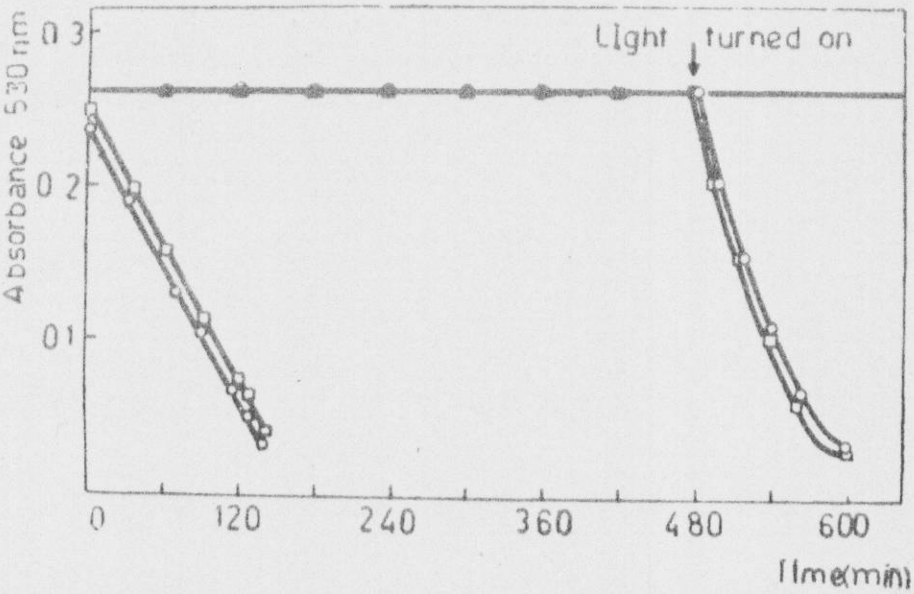

Fig. 11. Effect of riboflavin on auxin protector tinduced lag in IAA-oxidation in light and in dark. Riboflavin added in the presence of light (0), and in dark (●) ; riboflavin and auxin protector in light (□), and in dark (▪). Auxin protector (Pr-I, Pr-II and Pr-III separately) induced lag is abolished by riboflavin in presence of light.