Short Communication

INTERPRETATION OF THE AUXIN-SPARING MECHANISM ON THE BASIS OF FREE-RADICALS

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The effects of light on plant growth and morphogenesis have been reported by many workers. It has been assumed (Engelsma and Meijer, 1965; Galston, 1965; Bastin, 1966) that light control of phenolic compounds synthesis acts in growth regulation through a control of indoleacetic acid level. Since Galston, Bonner and Baker (1953) found that peroxidase catalyzes oxygen-consuming oxidation of IAA, the mechanism whereby diphenolic compounds inhibit the reaction has been investigated in respect to the enzyme-substrate complex theory (Rabin and Klein, 1957; Pilet, 1964; Bastin, 1964). On the other hand, indoleacetic acid can be photo-oxidized by light in the presence of riboflavine (Galston and Baker, 1949) but insufficient evidence has been presented that this photo-inactivation can be affected by the presence of phenolic compounds.

It is the objective of the present communication to suggest a fundamentally mechanism for enzymatic oxidation of indoleacetic acid and to explain the inhibitory effect of diphenolic compounds in the reaction by light and by peroxidase containing preparations.

As shown in Fig. 1, catechol, malvidin and hydronaphtoquinone inhibit IAA oxidation by light (Fig. 1a) in the presence of riboflavine and by horseradish peroxidase (Fig. 1b). At pH 4.45, 50% inhibition was obtained with $2 \times 10^{-6} \text{M}$ catechol and hydronaphtoquinone and with $6 \times 10^{-6} \text{M}$ malvidin. The same inhibition was reached with lower concentrations of effectors in the IAA-oxidizing activity of peroxidase. This inhibition was reduced by increasing indoleacetic acid concentration. In Fig. 2, Lineweaver-Burk plots of the results clearly show that the inhibition is competitive with respect to IAA.

Anthocyanin was useful for demonstrating the effect of diphenol in photo-oxidation of IAA. Fig. 3, plotted in Lineweaver Burk forms, details

\footnote{Abreviation used in this work: $\text{AH}_2$ = peroxidase substrate; $\text{DCP} = 2,4$-dichlorophenol; $\text{ES}_1$ = peroxidase-peroxide compound I; $\text{ES}_2$ = peroxidase-peroxide compound II; $\text{HNQ} = 2$-hydroxy-L4-dihydroxynaphtalene-4-glucoside; $\text{HO}_2$ = perhydroxyl free-radical; $\text{HRP} = \text{horseradish peroxidase}; \text{IAA} = \text{indoleacetic acid.}
the result obtained. There is a non-competitive inhibition exerted by malvidin in the photo-oxidizing system.

The data presented in Fig. 2 is in agreement with those already published (RABIN and KLEIN, 1957; BASTIN, 1964; PILET, 1964). This evidence supports the conclusion that IAA and peroxidase substrates react with a common catalytic center of peroxidase. Spectral changes in the enzyme were demonstrated upon the addition of IAA under aerobic conditions (YAMAZAKI and SOUZU, 1960; BASTIN, GASPAR and LEYH, 1965; FOX, PURVES and NAKADA, 1965; RICARD and NARI, 1966) and different mechanisms for IAA-oxidase reaction catalyzed by peroxidase were proposed.

According to YAMAZAKI and SOUZU (1960), in the iron-reducing activity of peroxidase in the presence of IAA as electron donor, the kinetic and stoichiometric evidence supports a free-radical mechanism in which peroxidase catalyzes the formation of 2 moles of IAA radicals and 1 mole of hydrogen peroxide. This mechanism was confirmed by HENMAN and LANG (1965) who proposed a reaction involving the initial extraction of an electron from IAA to form a free radical and a rapid reaction of the radical with molecular oxygen.

Evidence has been presented (BASTIN et al., 1965; FOX et al., 1965) that IAA can interact with HRP to form compounds spectrally similar to compounds I and II formed from HRP and simple peroxides.