THE DIRECT INVOLVEMENT OF HYDROGEN PEROXIDE IN INDOLEACETIC ACID INACTIVATION

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SUMMARY: Hydrogen peroxide appears to mask the chemical characteristics of indoleacetic acid. This was demonstrated by the Salkowski and Fluorescence tests. Stem elongation and root initiation were inhibited as a result of adding H_2O_2 to nutrient media containing IAA, however, upon the addition of purified catalase, most of the symptoms of IAA inactivation were reversed. It is suggested that in vivo IAA may be regulated partially by its conjugation with H_2O_2, and catalase may have a role in the IAA reactivation process. The accumulation of hydrogen peroxide in the cells as a result of catalase inhibition may lead to a temporary IAA inactivation, therefore effecting plant growth.

INTRODUCTION

Peroxides are widely recognized as toxic waste products of metabolism, they are located primarily in the microbodies (1) however, hydrogen peroxide formation by isolated mitochondria (2) and chloroplasts (3) has been reported. The removal of hydrogen peroxide is essential to prevent destructive oxidation from occurring. Catalase EC 1.11.1.6 and peroxidases EC 1.11.1.7 are capable of performing this function (4) Plants contain enzymes capable of oxidizing indoleacetic acid (5) and hydrogen peroxide may be involved in this oxidative breakdown. However, the possibility of a direct involvement of peroxides in the inactivation of IAA is still unresolved. This work describes the direct effects of Hydrogen peroxide on indoleacetic acid activities.

METHODS AND MATERIALS

Indoleacetic acid determination: IAA was determined by using Slakowiski reagent (6). A Bausch and Lomb spectronic 20

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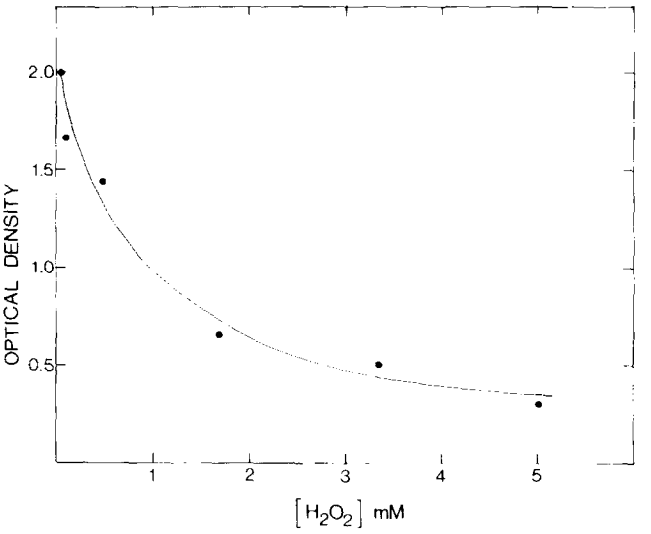

FIGURE I *The effect of hydrogen peroxide on indoleacetic acid. The incubation media contained H_2O_2, 1mM, IAA and 50mM phosphate-citrate buffer pH 6.0 the incubation time was 10 minutes. The amount of IAA was estimated with the Salkowski reagent.*

spectrophotometer set at a wave length of 540 nm was used. In the fluorescence experiments. Fluorescence intensities of solutions were determined in a Perkin-Elmer model 204 Fluorescence spectrophotometer. IAA exhibited an excitation maximum at 290 nm and fluorescence emission maximum at 360 nm.
Stem elongation experiment: Seeds of pea (Pisum sativum L. Var Century) were germinated in vermiculite in the dark at 25°C for 10 days. Just below the apical hook, 6mm segments were cut and 10 segments were immersed in petri dishes containing 2% sucrose. The dishes were stored in the dark at 25°C. Hydrogen peroxide and indoleacetic acid concentrations were 10mM and 5μM respectively. Purified catalase was added at the rate of 50μg/ml The length of the stem segments were measured every 24 hours.
Root initiation experiment: Seeds of beans (Phaseolus vulgaris L. var. Slendergreen) were grown in vermiculite at 25°C for 10 days in light. The seedlings were cut at the vermiculite level and surface sterilized by dipping the cut end in a 5% Clorox solution for 2-3 minutes. Four plants were immersed in a 250 ml Erlenmayer flask containing 200 ml one-fourth strength Hoagland's solution (7). H_2O_2, IAA and catalase concentrations were the same as described in the stem elongation experiment. Plants were incubated in a growth chamber set at 25°C and 16 hr light and 60-70% relative humidity. The number of roots was recorded every 24 hours.

RESULTS AND DISCUSSION

Effects of hydrogen Peroxide on indoleacetic acid.

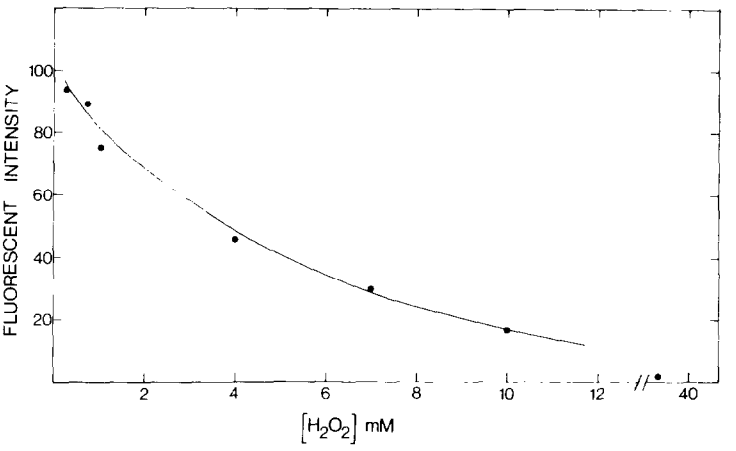

Figure II *Effect of hydrogen peroxide on the fluorescence of indoleacetic acid.*
The incubation media contained H_2O_2, 1μM, IAA and 50mM phosphate-citrate buffer pH 6.0. The incubation time was 10 minutes.

a) Salkowski test:

The inability of IAA to react normally to the Salkowski reagent as the level of hydrogen peroxide was increased is shown in Figure I. When H_2O_2 concentration reached 20mM, IAA was totally inactivated.

b) Fluorescence test:

The continuous reduction in the fluorescence of IAA as a result of increasing H_2O_2 concentration is shown in Figure II. When H_2O_2 concentration reached 30mM the fluorescence was at minimum. In these two experiments, when 50μg/ml commercially purified catalase was incubated or added to the H_2O_2 IAA mixture, IAA showed a positive Salkowski reaction and also the fluorescence was regained. It is possible that H_2O_2 forms a complex with IAA therefore masking its chemical characteristics (fluorescence and Salkowski test). Carboxylic acids when mixed with hydrogen peroxides are known to form peroxy acids in vitro (8).

Effects of hydrogen peroxide on the acitivty of IAA.

TABLE 1 The Effect of Catalase on the Inactivation of IAA By Hudrogen Peroxide

TREATMENT	AVERAGE INCREASE IN STEMS (mm)		NUMBER OF ROOTS FORMED	
	3 Days	6 Days	7 Days	10 Days
1. Control	0.8	0.9	47	53
2. Catalase	0.7	0.8	39	46
3. IAA	1.8	2.2	56	64
4. H_2O_2	0.1	0.1	16	16
5. H_2O_2*	0.1	0.8	16	35
6. IAA + H_2O_2	0.1	0.1	15	17
7. IAA + H_2O_2*	0.1	0.6	12	26
8. IAA - H_2O_2 + Catalase	2.2	2.9	49	58
9. Catalase + H_2O_2	0.6	0.8	41	50
10. Catalase + IAA	2.2	3.0	50	61

* Catalase was added after 3 days in the stem elongation experiment and after 7 days in the root initiation experiment.

1. Stem elongation

The average increase in length of stems after 6 days incubation with 5μM IAA was 2.2 mm while the incubation with 10mM H_2O_2 and 5μM IAA resulted only in an average increase of 0.1 mm (Table I).

2. Root initiation:

Table I shows the effect of hydrogen peroxide on root initiation. Treatment 4 where H_2O_2 was added to the nutrient media showed a sharp decline in the number of roots as compared to treatment 3 where IAA was added. In treatment 6 when H_2O_2 and IAA were added at the same time the number of roots did not change appreciably.

When 50μg/ml commercial purified catalase was added to the incub-

ation media after 3 days in the stem elongation experiment and 7 days in the root initiation experiment, IAA was reactivated (Table I). When catalase was added to treatment #7 there was an increase in the average stem elongation from 0.1 to 0.6 mm as compared to treatment #6 in which the stems showed no increase in elongation due to the absence of catalase. The same results can be seen in the root initiation experiment. When catalase was added after 7 days incubation with the H_2O_2 - IAA solution, the number of roots increased from 12 to 26 (treatment #7), in contrast to treatment #6 in which the number of roots increased from 15 to only 17 roots since no catalase was added. Treatment #8 showed no inactivation of IAA by H_2O_2 since catalase was present initially in the nutrient media of both experiments.

It is possible that H_2O_2 forms a complex with IAA, therefore masking its biological activity in promoting stem elongation and root growth. There are reports indicating the inactivation of IAA by its conjugation with a variety of compounds such as the formation of indole-3-acetyl aspartic acid and possibly indole-3-acetyl glutamic acid in excised tomato roots exposed to IAA (9), also the reported trace quantity of auxin-sugar conjugate (10). These complexes may inactivate IAA, however, it is not clear whether the conjugated IAA can be readily released.

In this study it appears that IAA was not destroyed by H_2O_2, instead probably an IAA-H_2O_2 complex may have been formed in which IAA was not active. When catalase was introduced, H_2O_2 was removed from this complex and IAA was reactivated again.

It is understood that in normal healthy plant tissues H_2O_2 is not accumulated in a significant amount. However several factors may effect the level of hydrogen peroxide in the cell. Patterson and Myers (11) have shown that high light intensity or cold

shock caused a sudden increase in the H_2O_2 level of Anacystis nidulans. Other types of disruptions may occur when a specific inhibitor affects catalase and not for example glucose oxidase. Cu^{++} (single or in combination with ascorbate) is an example of a selective inhibition that may result in an accumulation of H_2O_2 in the cell (12). Also growing plants under unfavorable environmental conditions such as chilling temperature may cause some damage to the cell membranes and the leakage of electrolytes (13). When bean seedlings were exposed to 5°C for 24 hours, there was a drop in catalase activity accompanied by an increase in the peroxide level in the leaves (Omran and Eisen, unpublished data). Even in healthy tissues, although the peroxides activities are confined mainly to the microbodies, Halliwell (1) did not exclude the possibility of peroxide diffusion from the microbodies. This allows other metabolites in the cytoplasm including IAA to react with hydrogen peroxide. In view of these findings, it is suggested that IAA may be regulated, to some degree, by its inactivation when complexed with H_2O_2 and its reactivation upon the removal of hydrogen peroxide. Such complexation could lead to the inhibition of IAA transport. Plant tissues or cells that exhibit low catalase activity and/or high H_2O_2 production would tend to show low IAA activity. Other areas of the plant which have low H_2O_2 level and/or high activity of catalase would tend to show normal IAA activity.

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