Studies on the Auxin Metabolism of Some Cereal Crops in Relation tO Growth and Development I. Some Characteristics of the Indoleacetic Acid-oxidizing Enzyme in Wheat Seedlings

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Studies on the Auxin Metabolism of Some Cereal Crops in Relation to Growth and Development

I. Some Characteristics of the Indoleacetic Acid-oxidizing Enzyme in Wheat Seedlings[1]

Jiro HARADA and Kiyoshi WADA

Department of Agronomy, Faculty of Agriculture
Tohoku University, Sendai, Japan

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Summary

Some characteristics of IAA-oxidizing enzyme in dark-grown wheat seedlings were studied and the results obtained were summarized as follows;

After demonstrating the existence of IAA-oxidizing system in seedlings, the crude and dialysed homogenates were prepared from root, coleoptile and plumule. Then, their IAA-oxidizing and guaiacol-peroxidizing activities were determined and compared with each other.

The dialysed enzyme solution showed a considerably high IAA-oxidizing activity. Descending order of this activity is as follows: root>coleoptile> plumule; pH 4.5, 5.0 and 5.5 were the optimum pH values in root, coleoptile and plumule, respectively. Similar results were obtained in the activity of peroxidase from the same source.

There was, however, an eminent difference between the crude and dialysed enzyme solution in the mode of action in that the crude enzyme solution is lacking in IAA-oxidizing activity in spite of its high peroxidase activity. It may be concluded, therefore, that the lack of IAA-oxidizing activity of the crude enzyme is due to the existence of some dialysable, heat-stable inhibitors in the solution.

Furthermore, in order to clarify the characteristics of IAA-oxidizing enzyme, the authors have undertaken some experiments and obtained the following results.

IAA-oxidizing activity of the dialysed enzyme solution was inactivated by heat treatment in exactly the same way as the activity of peroxidase in the same solution; it was promoted by the addition of DCP and Mn^{++}.

In the mode of inhibitory action of some enzyme inhibitors added, there were considerable differences between IAA-oxidiation and guaiacol-peroxidation. A comparatively low concentration, 10^{-5} M of H_2O_2, showed little promotion of IAA-oxidizing activity in the dialysed enzyme but the activity was greatly inhibitied by a much higher concentration, 10^{-4} M of H_2O_2, and catalase showed little effect on the activity of the same solution.

From the above mentioned results, it may be concluded that IAA-oxidizing enzyme in dark-grown wheat seedlings is a peroxidase and there are some differences

[1] A portion of the thesis for the Master of Science degree submitted by the senior author to the Graduate School, Tohoku University.

between IAA-oxidiation and guaiacol-peroxidation in the reaction mechanism. Furthermore, from the results concerning the effects of catalase and H_2O_2, it is suggested that IAA may be oxidized by wheat seedling peroxidase through the same reaction mechanism as the scheme presented by Yamazaki, et al (1960).

It is well known that growth and development in plants are closely related to the auxin level. The auxin level may be directly controlled through the balance of its synthesis and destruction. From this viewpoint, the authors have been studying the auxin metabolism of some cereal crops.

The present paper is a partial result of these experiments conducted for clarifying the characteristics of IAA[2]-oxidase in wheat seedlings.

Material and Methods

Material

The wheat seeds,[3] variety "AOBAKOMUGI," were soaked for five hours, and then placed in petri-dishes with two layers of wet filter paper and kept in the dark at 24°C for four days. These dark-grown seedlings were used for this experiment.

Preparation of enzyme

The coleoptiles, plumules and roots were detached from the seedlings and were homogenized separately in a mortar with a small quantity of quartz sand and four parts of deionized water. The homogenates were filtered through two layers of cheese cloth and centrifuged at 2000×G for five minutes. After centrifuging, the supernatant was diluted with deionized water and this was used as the "crude enzyme solution." The "dialysed enzyme solution" was prepared by dialysis of the crude enzyme using "Visking cellulose tubing (6.4 m/m)" in 200 parts of deionized water at 0°–5°C for 48 hours. In both cases, 1 ml of enzyme solution was prepared from 0.2 g of fresh materials.

Measurement of enzyme activity

The peroxidase activity was measured by the guaiacol method of Kondo and Morita (1), while the IAA-oxidase activity was measured by the application of Salkowski's colorimetric method (2); that is, 5 ml of reaction mixture containing 3 ml (2 ml in the case of Figs. 5, 6) of buffer solution, 1 ml of enzyme solution and IAA giving a final concentration of 10^{-4} M (2×10^{-4} M in Figs. 5, 6) incubated at 30°C, shaking 100 times per minute for 60 minutes (30 minutes in Figs. 5, 6). After incubation, the amount of IAA in the reaction mixture was determined by the method mentioned above. The activity was expressed as the amount of IAA

[2] The following abbreviations will be used: IAA, indoleacetic acid; DCP, 2,4-dichlorophenol.

[3] The seeds were kindly supplied by Miyagi-ken Agricultural Experiment Station and Miyagi Agricultural College.

destroyed per 1 ml of reaction mixture. McIlvaine's (1/10 M citric acid–1/5 M NaH_2PO_4) buffer solution (pH 5.0) was used for this experiment except in some cases mentioned hereafter.

Results and Discussion

1. *Existence of IAA-destroying system.* One cm sections were cut separately from the base and tip of the coleoptile of 4-day-old dark-grown wheat seedlings. The ten sections were incubated with 5 ml of 30 ppm IAA solution, at 30°C in the dark and the remaining IAA was measured by the method mentioned above. The result is shown in Fig. 1; that is, after a lag period of 6 hours, the IAA decreased

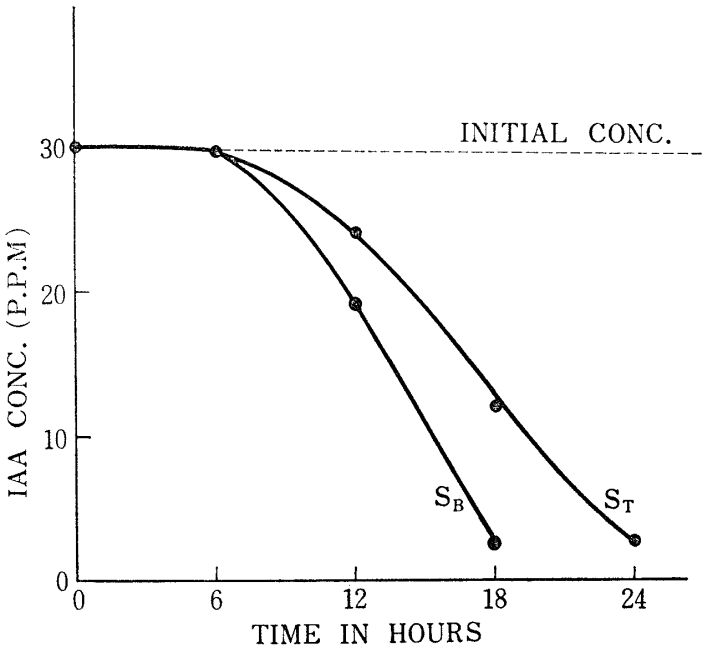

FIG. 1. IAA disappearance from solutions containing wheat coleoptile sections.
S_T: 1 cm section from tip. S_B: 1 cm section from base.

gradually and almost disappeared after 20 hours in the base sections and after 24 hours in the tip sections. The sections were taken every 6 hours, homogenized with reaction mixture and extracted with peroxide-free ether. The extracts were concentrated in vacuo and then used for paper chromatographic analysis using the developing solvent (iso-propanol: 28% ammonia: water 10:1:1 by volume) and Salkowski, Ehrlich[4] and Aldehyde[5] reagents as a detecting reagent.

The result (Table 1), showing that indolealdehyde increased gradually accord-

[4] Solution of p-dimetylaminobenzaldehyde (2g) in the mixture of conc. HCl (10 ml) and 95% ethyl alcohol (10 ml).

[5] Saturated solution of 2,4-dinitrophenylhydrazine in 2N HCl.

TABLE 1. *Change of Indoleacetic Acid to Indolealdehyde Induced by Wheat Coleoptile Sections.*

	Rf*	Color reaction			Incubation period (*hours*)		
		Salkowski	Ehrlich	Aldehyde	0	12	24
Indoleacetic acid	0.56	pink	reddish blue	—	|||	|||	–
Indolealdehyde	0.95	—	brown	yellow	+	|||	|||

* Iso-propanol: 28% ammonia: water (10:1:1 by volume) was used as the developing solvent.

ing to the decrement of IAA, indicated the existence of IAA-destroying system in wheat coleoptiles.

2. *Optimum pH.* Effects of the pH on the IAA-oxidation and guaiacol-peroxidation were measured using the dialysed enzyme solution from different sources. The result (Fig. 2) showed that the descending order of IAA-oxidizing activity is as follows: root>coleoptile>plumule. pH 4.5, 5.0 and 5.5 were the optimum pH values in the root, coleoptile and plumule, respectively. Similar results were obtained in the activity of peroxidase from the same source.

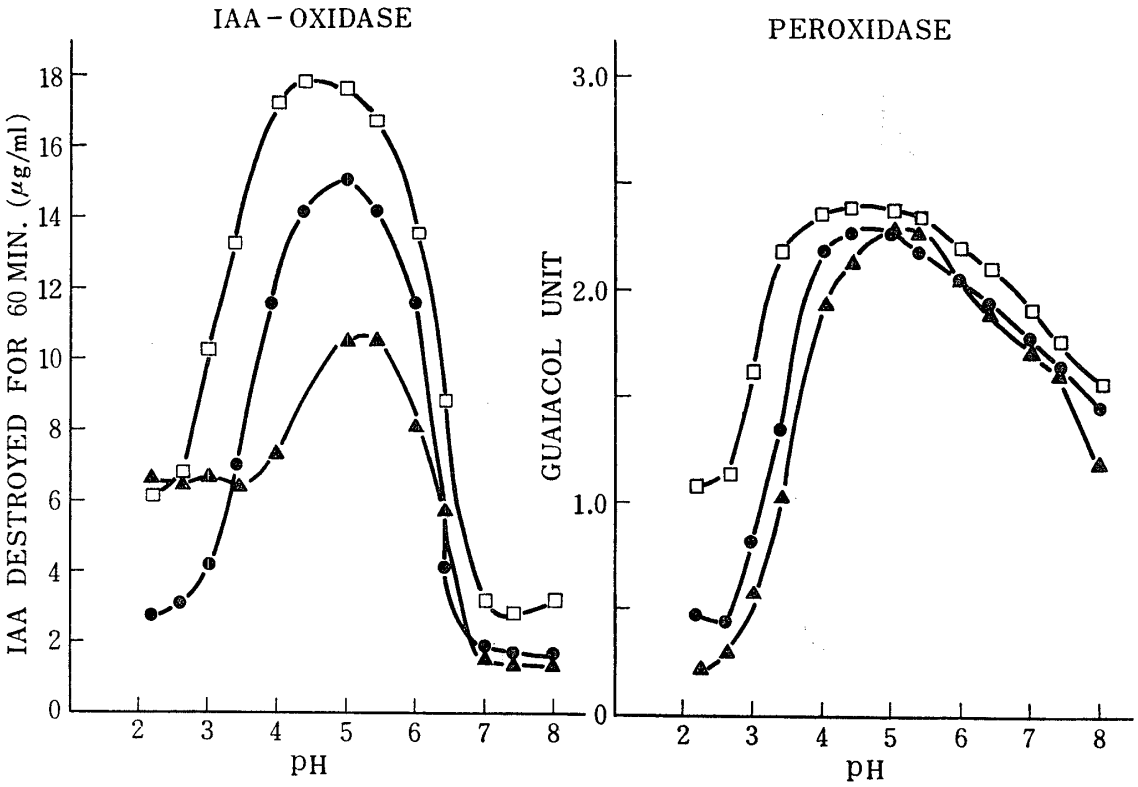

FIG. 2. The effect of pH on IAA-oxidase and peroxidase activities of the dialysed enzyme solution.

-□- root -●- coleoptile -▲- plumule

It has been reported that the optimum pH value of IAA-oxidase is widely different. For example, 6.2–6.7 in pea seedling (3), 6.0–6.5 in bean root (4), 6.2–

6.5 in rice root (5), 4.0 in rice coleoptile (6), and 3.5 in pineapple (7) and *Omphalia flavida* (8) was reported.

Wada (6) considered that these differences did not depend on the quantitative or qualitative differences of inhibitors and co-factors existing in plant tissues, but on the characteristics of the enzyme itself.

From the present data which show the similarity of optimum pH between IAA-oxidase and peroxidase, it is suggested that IAA-oxidizing enzyme may be a peroxidase and the difference of its optimum pH in plant tissue may be based on the difference of each component of isozyme.

3. *Existence of IAA-oxidase inhibitor.* Using the crude and dialysed enzyme solution from coleoptile, IAA-oxidizing and guaiacol-peroxidizing activities were determined and compared with each other. As a result (Fig. 3), there was an eminent difference between them in the mode of action, in that the dialysed enzyme solution showed considerably high IAA-oxidizing and guaiacol-peroxidizing activities while the crude enzyme solution lacked in IAA-oxidizing activity in spite of its high peroxidase activity.

IAA-oxidase activity of the dialysed enzyme was perfectly inhibited by adding boiled extract of wheat seedling (Fig. 3).

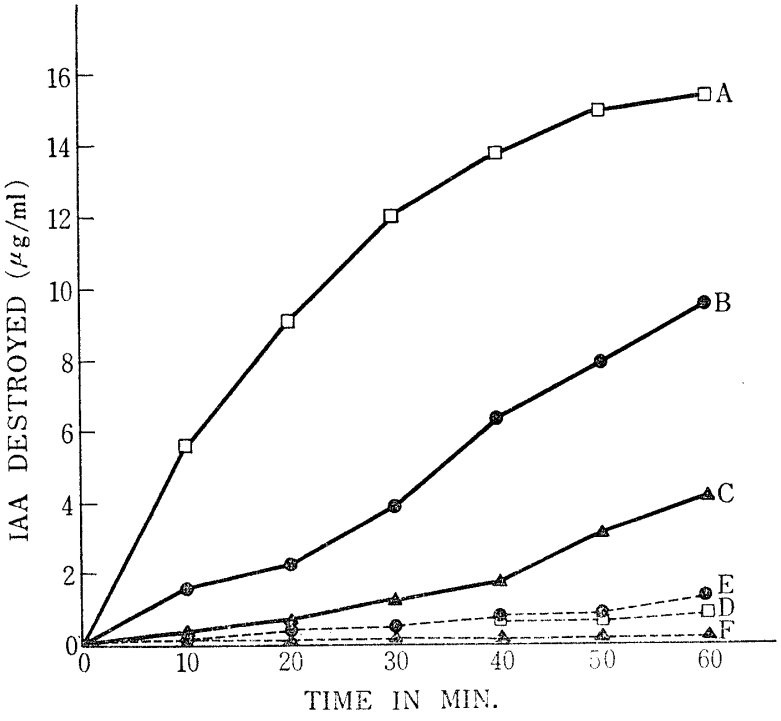

FIG. 3. The kinetics of IAA-oxidation by the crude and dialysed enzyme solutions and the effect of added boiled extract of wheat seedlings.

A: root (dialysed), B: coleoptile (dialysed), C: plumule (dialysed), D: root (crude), E: coleoptile (crude), F: plumule (crude) or coleoptile (dialysed)+boiled extract (20 mg fresh weight/ml).

It may be, therefore, concluded that lack of IAA-oxidizing activity of the crude enzyme was due to the existence of some dialysable heat-stable inhibitors in the solution.

Ojima (9) indicated the existence of some IAA-oxidase inhibitors in wheat root; chlorogenic acid (7, 10, 11), caffeic acid (10), ferulic acid (12) and flavonol complex (13) have been reported as a naturally occurring inhibitor of IAA-oxidase and the physiological role of these substances will be clarified soon.

4. *Effect of heat treatment.* IAA-oxidizing activity of the dialysed enzyme solution from coleoptile was inactivated by heat treatment in exactly the same way as the activity of peroxidase in the same solution was inactivated (Fig. 4). The

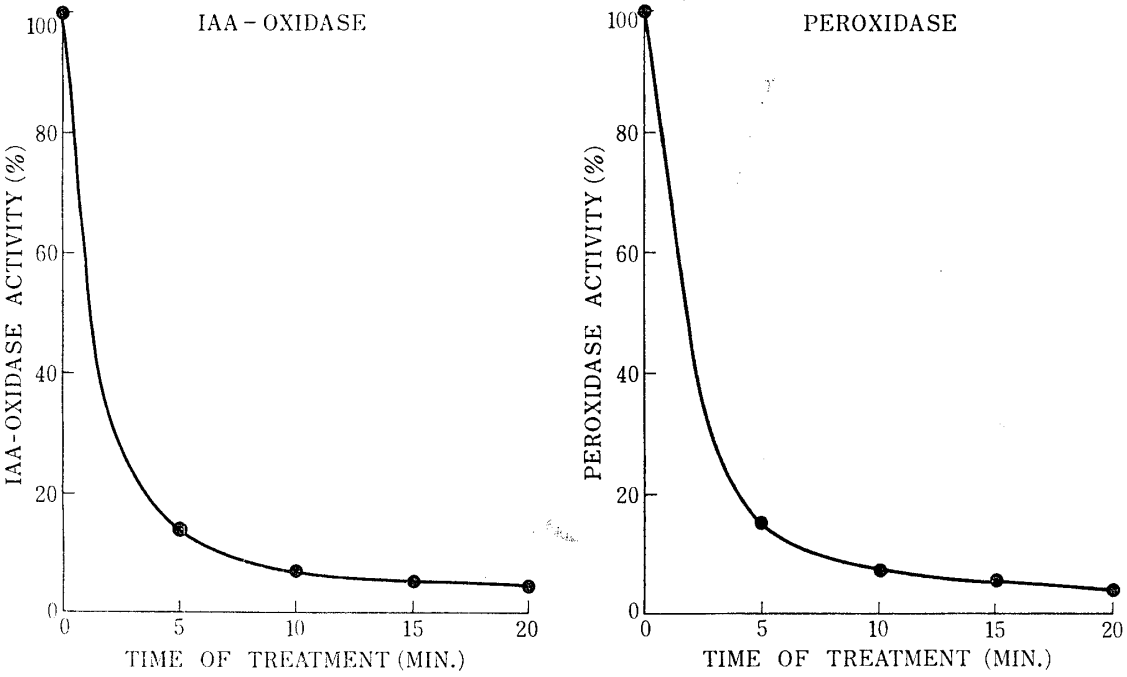

FIG. 4. The effect of heat treatment (100°C) on IAA-oxidase and peroxidase activities of the dialysed enzyme solution.

same results were obtained in the enzyme from plumule and roots. It has been reported that the IAA-oxidizing enzyme existing in plant tissues is mostly a peroxidase (14) except in some cases of tyrosinase (15) or catalase (16).

From the results obtained, it may be concluded that IAA-oxidase of dark-grown wheat seedling is a peroxidase, as has been the general findings.

5. *Effect of DCP and* Mn^{++}. It is well known that IAA-oxidase activity is promoted by the addition of Mn^{++} and DCP (17), but some workers have reported the inhibitory action of Mn^{++} due to the chelating effect of buffer used (18, 19). In order to clarify this point, the effect of Mn^{++} on IAA-oxidase of wheat coleoptile was examined using different buffer solutions, and the following results were obtained (Fig. 5).

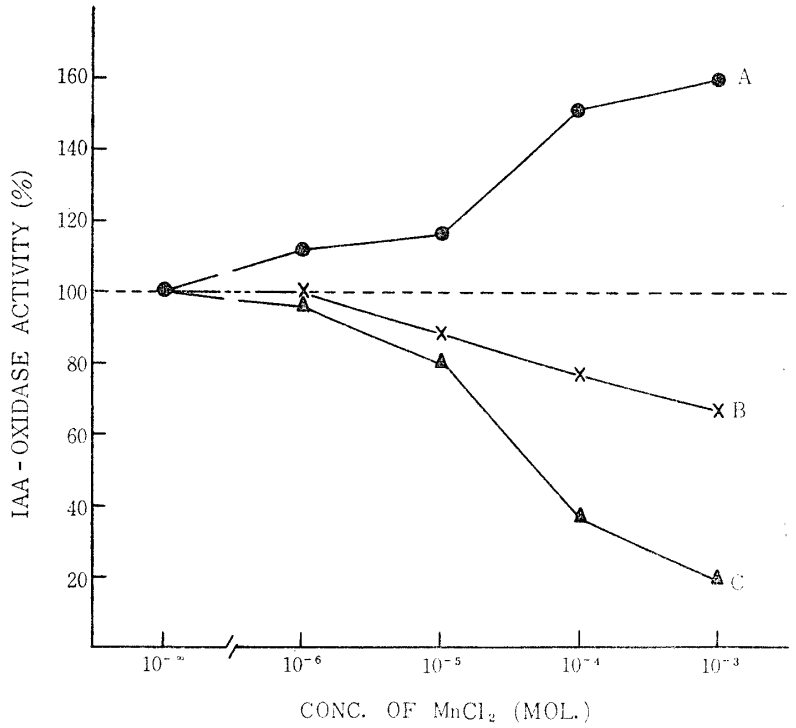

FIG. 5. The effect of $MnCl_2$ on IAA-oxidase of the dialysed enzyme solution using different buffer solutions.
A: Michaelis' (1/10 N Na_2HPO_4-1/10 N KH_2PO_4) buffer (pH 5.2)
B: Walpole's (1/5 M Acetic acid—1/5 M CH_3COONa) buffer (pH 5.0)
C: McIlvaine's (1/10 M Citric acid—1/5 M Na_2HPO_4) buffer (pH 5.0)

IAA-oxidase was promoted by the addition of Mn^{++} only in the case of phosphate buffer solution being used, and was inhibited in others. This promotion was accelerated by further addition of DCP (Fig. 6). These results suggest the importance of buffer solution in determining IAA-oxidase activity.

However, in the case of acetate buffer which showed the inhibitory effect in the present experiment, Wada (6) could not find any inhibitory action in rice coleoptile enzyme. Also, it has been reported that IAA-oxidase obtained from etiolated pea epicotyls was inhibited by the addition of Mn^{++} although phosphate buffer was used (4). So, it will also be possible to consider that the effect of Mn^{++} is varied among the enzyme from different sources.

6. *Effects of enzyme inhibitors.* Table 2 shows the effects of some enzyme inhibitors on IAA-oxidation and guaiacol-peroxidation by the dialysed enzyme solution from wheat coleoptiles. The activities of both enzymes were greatly inhibited by NaN_3 and KCN, and considerably by thiourea. Monoiodoacetic acid, known as SH-enzyme inhibitor, slightly inhibited peroxidase activity and promoted IAA-oxidase. IAA-oxidation was strongly inhibited by sodium diethyldithiocarbamate, a sort of copper-enzyme inhibitor while peroxidase activity was not at all

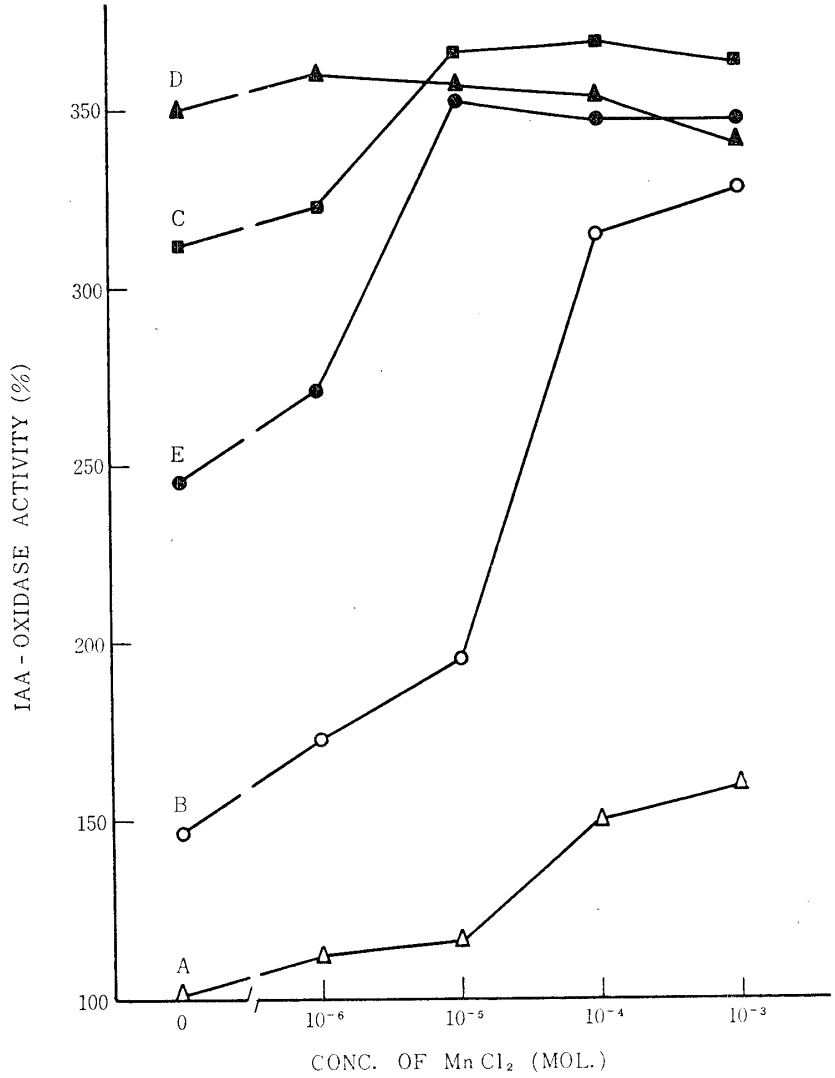

FIG. 6. Effects of DCP and $MnCl_2$ on IAA-oxidase of the dialysed enzyme solution. (Using buffer: Michaelis' buffer solution, pH 5.2). Conc. of DCP (Mol.) A: 0, B: 10^{-6}, C: 10^{-5}, D: 10^{-4}, E: 10^{-3}.

TABLE 2. *Effects of Some Enzyme Inhibitors on IAA-oxidation and Guaiacol-peroxidation by the Dialysed Enzyme Solution.*

	Inhibition (%)	
	IAA-oxidation	Guaiacol-peroxidation
NaN_3 (10^{-3} M)	76.0	87.1
α·α'-Dipyridyl (10^{-3} M)	5.1	7.1
Na-Diethyldithiocarbamate (10^{-3} M)	88.0	0.0
Thiourea (10^{-3} M)	56.0	17.8
8-Hydroxyquinolin (10^{-4} M)	15.1	0.0
EDTA (10^{-3} M)	23.1	3.5
Monoiodoacetic acid (10^{-3} M)	*	3.5
KCN (10^{-3} M)	88.0	91.8

* Slight promotion was observed.

affected by the same inhibitor. The effects of other inhibitors tested were considerably slight. It had been discussed that IAA-oxidase is an iron-enzyme or a copper-enzyme (3, 4), but Wada (6) reported that sodium diethyldithiocarbamate inhibited the IAA-oxidation by turnip crystalline peroxidase, which is evidently an iron-enzyme.

From this finding, it may be concluded that there are some differences between IAA-oxidation and guaiacol-peroxidation in the reaction mechanism in spite of IAA-oxidase being a peroxidase itself.

7. *Effects of catalase and* H_2O_2. In order to clarify the differences between IAA-oxidation and guaiacol-peroxidation, the effects of catalase and H_2O_2 on the IAA-oxidation were tested and the following results were obtained (Figs. 7, 8); that is, the IAA-oxidation was inhibited by the addition of beef liver catalase. The same inhibition was observed even though the inactivated catalase was added to a reaction mixture, while dialysed catalase showed little effect on the IAA-oxidation. From these results (Fig. 7), it is considered that the inhibiting effect of

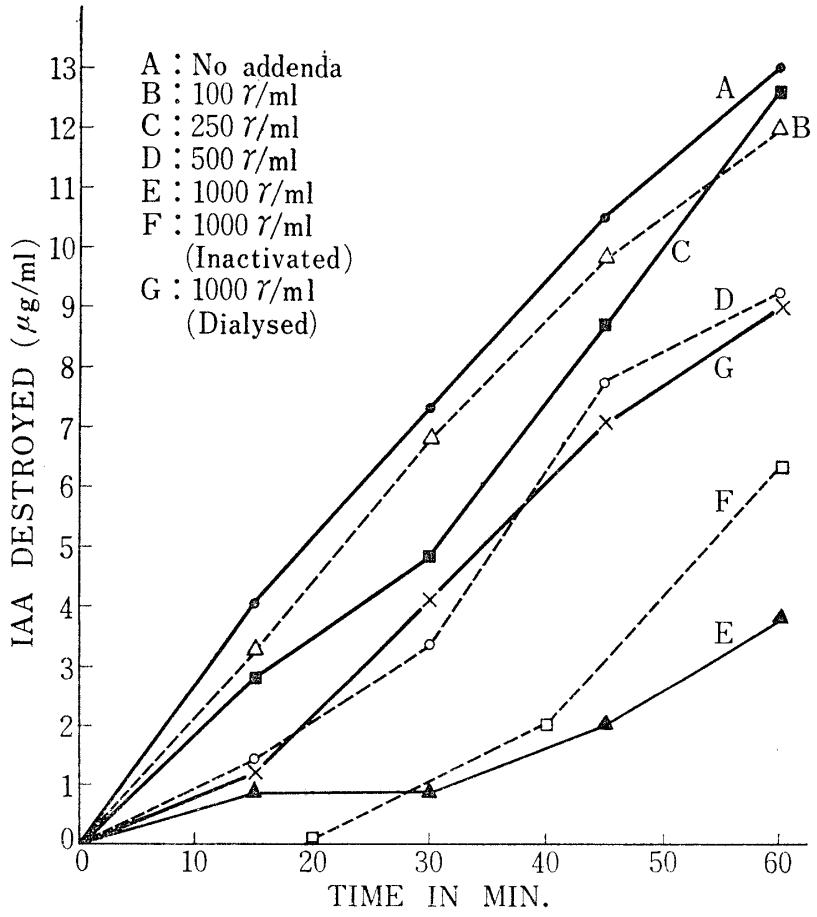

FIG. 7. The effect of beef liver catalase (Kat. f. =1500) on kinetics of IAA-oxidation by the dialysed enzyme solution. Catalase was inactivated completely by heating in a boiling water bath for 30 minutes, and was dialyzed with cellulose tubing in 200 volumes of deionized water for 48 hours at 0—5°C.

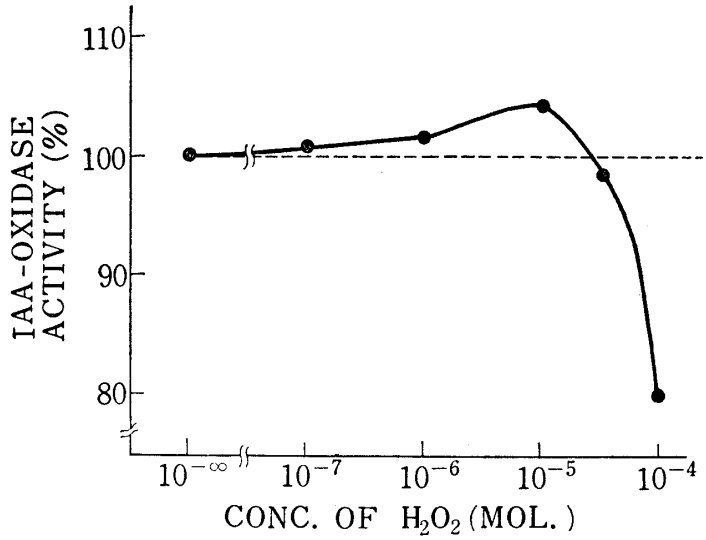

FIG. 8. The effect of H_2O_2 on IAA-oxidase activity of the dialysed enzyme solution. The reaction mixture with the inactivated enzyme was used as control because of interference of H_2O_2 with Salkowski's color reaction.

catalase used is not caused by catalase itself but by heat-stable, dialysable inhibitors mingling to an enzyme preparation.

A considerably low concentration, 10^{-5} M of H_2O_2, showed little promotion of IAA-oxidizing activity but the activity was greatly inhibited by a much higher concentration, 10^{-4} M of H_2O_2 (Fig. 8).

From the above mentioned results, it is suggested that IAA is oxidized by a wheat peroxidase through the same reaction mechanism as turnip crystalline peroxidase proposed by Yamazaki, et al (20); that is, the oxidation mechanism of IAA is different from the guaiacol-peroxidation in the point that the exogenous H_2O_2 is not needed as hydrogen acceptor because it is produced by the autoreduction of molecular oxygen. And so, the inhibition of IAA-oxidation caused by an excess of H_2O_2 may depend on "law of mass action" or the inactivation of enzyme caused by H_2O_2 (6).

Acknowledgement

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