Studies on the Physiological Effect of Gibberellin (V) : Effect of Gibberellic Acid and Gibberellin A on the Activity of Indoleacetic Acid Oxidase

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Studies on the Physiological Effect of Gibberellin

V. Effect of Gibberellic Acid and Gibberellin A on the Activity of Indoleacetic Acid Oxidase

By

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While the senior author (10, 11) holds an idea that the action mechanism of gibberellin differs from that of auxin, some other authors (7, 19, 20, 22) postulate that the growth promoting effect of gibberellin rests on its effect of increasing the auxin content of the tissue. Against the latter possibility, BRIAN and HEMMING (4) found that gibberellic acid had no effect on the activity of indole-3-acetic acid oxidase. The same result has been independently obtained by the present authors (12). Detailed descriptions of the experiments are reported here. The abbreviations, GA, GB and IAA stand for gibberellic acid[1], gibberellin A[2], and indole-3-acetic acid, respectively.

Material and Method

Seedlings of Usui peas (*Pisum sativum* L.) were used as the material for preparing IAA-oxidase. They were grown in a darkroom at 25°C for 7 days. Epicotyls were ground with a minimal volume of distilled water using Waring blender in the cold. The resulting brei was filtered through gauze, coarse fragments were removed by centrifuging briefly at about 2,300×g, and two volumes of cold acetone were added to five volumes of the supernatant. The precipitate obtained by centrifuging at about 9,000×g was suspended in half the original brei volume of cold phosphate-citrate buffer solution, pH 6.6. Cold acetone was added again to this suspension in the same proportion as above, and the precipitate obtained by centrifuging was resuspended in the cold buffer solution equivalent to 1/5 volume of the initial brei. The suspension was centrifuged, and the clear supernatant was used as the enzyme solution.

IAA-oxidase activity was determined by the decrease in intensity of the

1) Provided through the courtesy of Dr. C. LEBEN, Eli Lilly Co.
2) Provided through the courtesy of Prof. Y. SUMIKI, Tokyo Univ.

Salkowski reaction modified by GORDON and WEBER (8). Reaction mixture was maintained in a darkroom at 30°C to avoid inhibition of color development by light (5). The reaction was assayed by absorbancy at 530 mμ. As preliminaries, the effect of gibberellin on the Salkowski reaction was tested, since PLATT and THIMANN (18) have reported that the color development is inhibited in the presence of reducing agents and polyphenols. Solutions of GA and GB were added to IAA solution, the final concentration of IAA being 25 μg/ml and those of GA or GB, 1, 10, 50 and 100 μg/ml. GORDON and WEBER's reagent was added to these solutions and the color development was measured after 30 minutes of incubation.

Table 1. Effect of GA and GB on the color development of IAA by Salkowski reaction, measured by absorbancy at 530 mμ.

Concentration of GA or GB μg/ml	Absorbancy, %	
	GA	GB
0	100	100
1	100	100
10	99	99
50	94	98
100	88	96

Results as shown in Table 1 and Figures 1 and 2 show that both GA and GB inhibit the color development only slightly even in high concentrations, GB being less effective than GA, and that the shape of the absorption curves was not significantly altered. It was also confirmed that GA and GB themselves were negative in Salkowski reaction.

Results

Effect of GA and GB on IAA-oxidase activity.

The enzyme solution was added to solutions, IAA, IAA+GA, and IAA+GB. The final concentrations of IAA, GA and GB were 25, 10 and 10 μg/ml, respectively, the enzyme solution being diluted by a factor of 5. The volume of each reaction mixture was 4 ml. They were incubated at 25°C and samples were withdrawn after 0.5, 1, 2, 3 and 5 hours to determine their IAA content. As shown in Figure 3, IAA-oxidase activity was not affected by the presence of 10 μg/ml of GA and GB.

Effect of concentration of GA and GB on the IAA-oxidase activity.

Test solutions were made up by mixing IAA, GA or GB, and the enzyme solution together so that the final concentration of IAA was 25 μg/ml, and that of GA or GB, 1, 10, 50 and 100 μg/ml. They were incubated at 25°C and samples were taken after 1, 3 and 5 hours to measure the remaining IAA. Correction was made for the inhibition of Salkowski reaction by high concentrations of GA and GB, referring to Table 1. And the results presented in Tables 2 and 3 were obtained. It is shown that GA and GB have no effect on the IAA-oxidase activity in any concentration used.

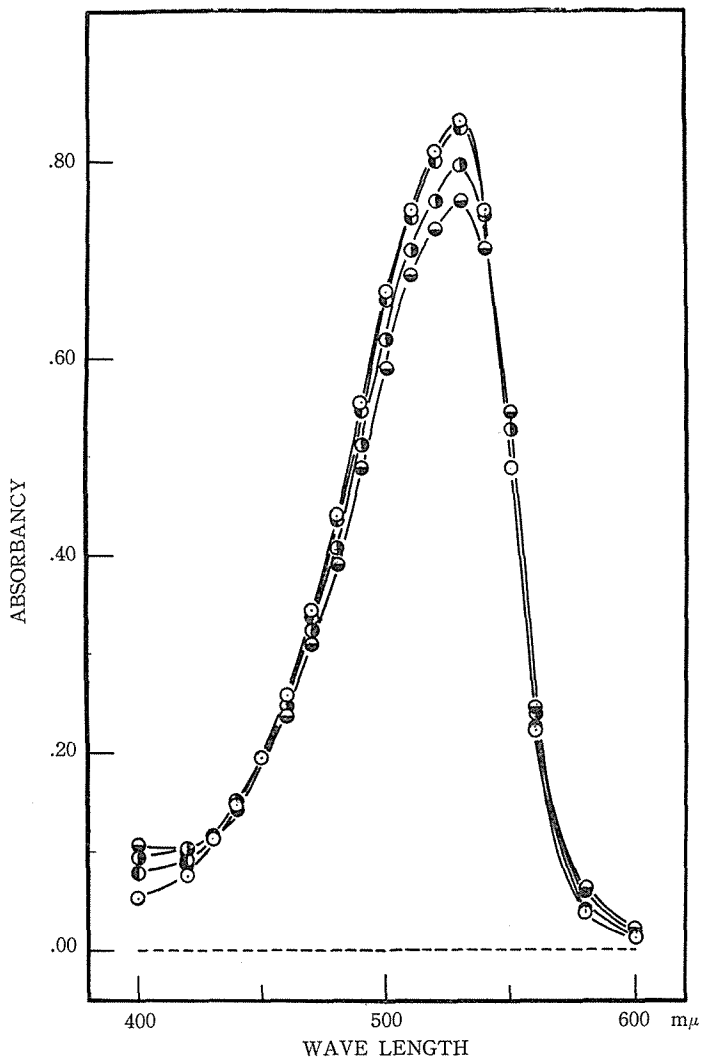

Fig. 1. Effect of GA on the color development of Salkowski reaction.

○——○ 25 μg/ml IAA,
●——● 25 μg/ml IAA+1 μg/ml GA,
◐——◐ 25 μg/ml IAA+10 μg/ml GA,
◑——◑ 25 μg/ml IAA+50 μg/ml GA,
◒——◒ 25 μg/ml IAA+100 μg/ml GA.

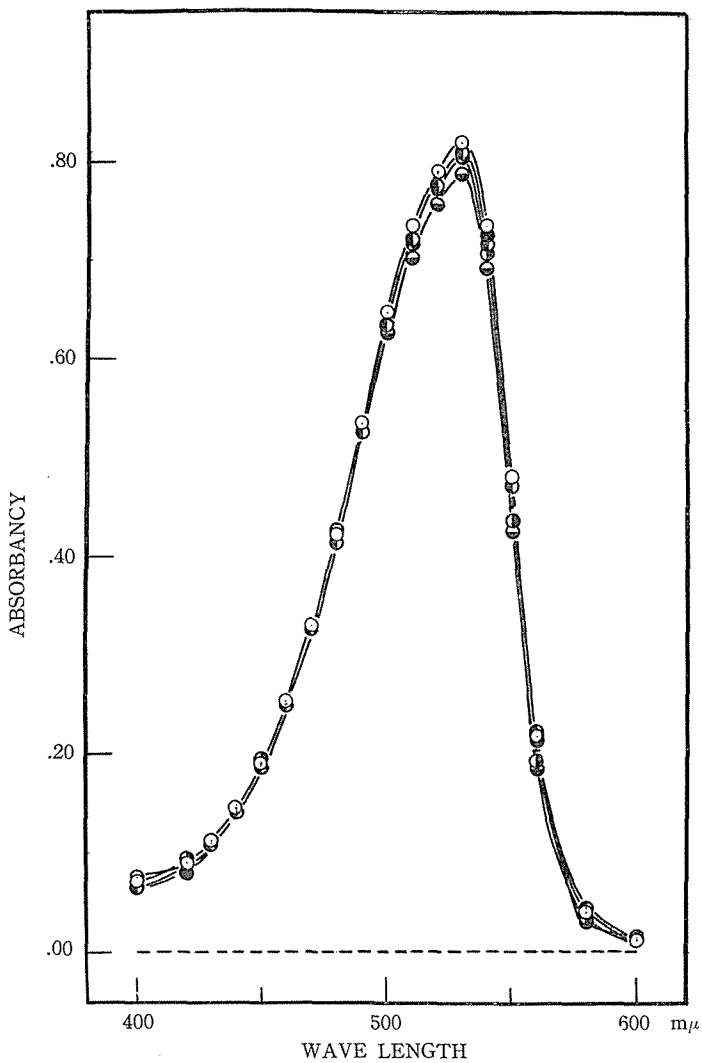

Fig. 2. Effect of GB on the color development of Salkowski reaction.

○——○ 25 μg/ml IAA,
◉——◉ 25 μg/ml IAA+1 μg/ml GB,
◐——◐ 25 μg/ml IAA+10 μg/ml GB,
◑——◑ 25 μg/ml IAA+50 μg/ml GB,
◒——◒ 25 μg/ml IAA+100 μg/ml GB.

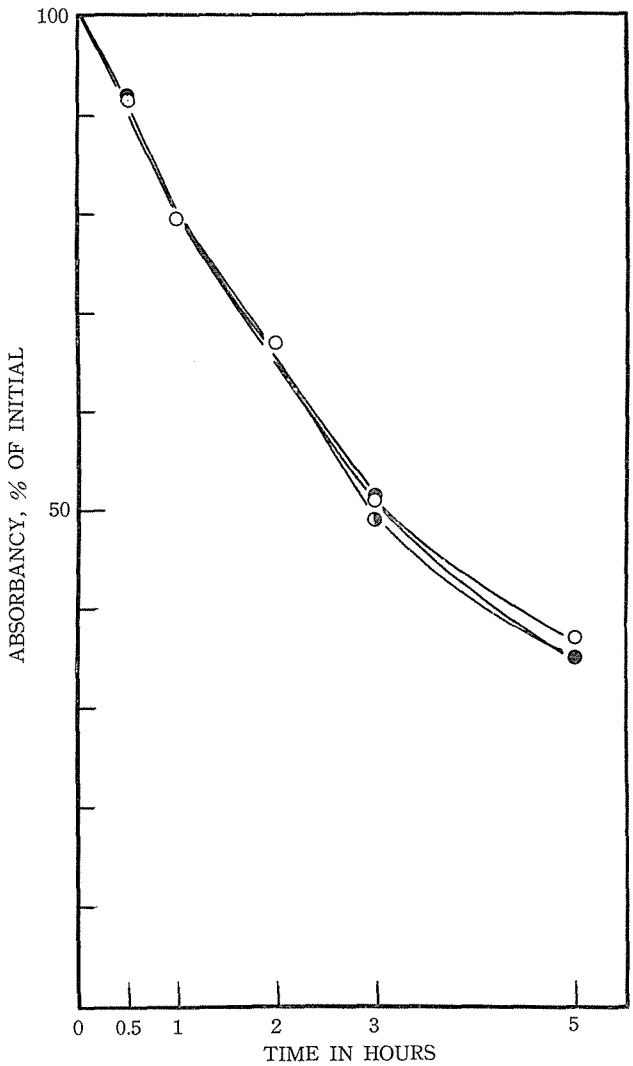

Fig. 3. Effect of GA and GB on the decrease of IAA in the presence of IAA-oxidase.

○——○ 25 μg/ml IAA+Enzyme,
●——● 25 μg/ml IAA+Enzyme+10 μg/ml GA,
◑——◑ 25 μg/ml IAA+Enzyme+10 μg/ml GB.

Table 2. Effect of concentration of GA on IAA-oxidase activity. Relative amount of IAA remaining undecomposed as represented by absorbance at 530 mμ by Salkowski reaction in percentage of that at zero time.

Time in hours	Concentration of GA, μg/ml				
	0	1	10	50	100
1	83	83	83	85	86
3	72	72	71	73	74
5	58	60	58	60	60

Table 3. Effect of concentration of GB on IAA-oxidase activity. Relative amount of IAA remaining undecomposed as represented by absorbance at 530 mμ by Salkowski reaction in percentage of that at zero time.

Time in hours	Concentration of GB, μg/ml				
	0	1	10	50	100
1	86	87	88	87	85
3	73	74	73	72	72
5	62	62	62	62	59

Effect of pH on the effect of GA on the IAA-oxidase activity.

The enzyme solution was prepared using distilled water at the final step, and was added to IAA+GA solutions which were adjusted to pH values from 3 to 8 by phosphate-citrate buffer. Samples were withdrawn after 1.5 and 3 hours of incubation at 25°C.

As the color development of Salkowski reaction differs according to pH, the correction factor for each pH was determined with the absorbance at pH 6.6. The results show that GA does not affect the enzyme activity in the pH range from 3 to 8.

Table 4. Effect of GA (10 μg/ml) on IAA-oxidase activity at various pH. Absorbancy is expressed as percentage of the initial absorbancy of the reaction mixture at pH 6.6.

pH	After 1.5 hours of incubation		After 3 hours of incubation	
	Control	GA	Control	GA
3.0	70	70	61	62
4.0	70	69	64	64
5.0	79	77	68	68
6.6	86	85	76	76
8.0	95	95	95	94

Effect in vivo of GA on the IAA-oxidase activity.

When etiolated pea seedlings, grown in a darkroom at 25°C, reached 4-5 cm in length, they were uniformly sprayed once a day with 10 μg/ml GA solution for two days. The next day epicotyls of sprayed and control seedlings were harvested, crushed and enzyme preparations were obtained as described above. The enzyme

solutions were added to IAA solution, of which the final concentration was 25 μg/ml, and incubated at 25°C for 3 hours. The results indicated that the GA application on living tissues had no effect on the IAA-oxidase activity measured after extraction.

Discussion

In the above-mentioned experiments we have observed that GA and GB had no stimulatory nor inhibitory effects on the activity of IAA-oxidase prepared from etiolated Usui pea seedlings. This result agrees exactly to BRIAN and HEMMING's result (4), and is also supported by MURAKAMI's result (15) obtained by manometric method. Moreover, no difference between the activity of IAA-oxidase from GA-treated seedlings and that from non-treated ones was found.

PILET (19) and PILET and WURGLER (20), using carrot and *Trifolium ochroleuchum* respectively, showed that gibberellin inhibited the IAA-oxidase activity *in vitro* and *in vivo*, and insisted that gibberellin action should be due to a change of auxin content in plant tissues. The same hypothesis had been suggested by STUTZ and WATANABE (22). GALSTON (7) observed that the treatment of pea seedlings with 10^{-4} M GA brought about higher level of IAA-oxidase inhibitor than in non-treated ones and postulated that the GA action might involve the sparing of auxin. On the other hand, HAYASHI and MURAKAMI (9) reported that in using several green plants no difference in auxin content was found between the gibberellin-treated material and the non-treated one.

VAN OVERBEEK *et al.* (23) demonstrated that IAA inhibits GA-induced elongation of *Avena* leaf sections. KUSE (13), using petioles of young sweet potato stems, postulated the necessity of auxin for the growth effect of gibberellin. It can be considered that the report of the former and that of the latter might be due respectively to a supra-optimal and the optimal concentration of auxin in the tissues, if the hypothesis represented by PILET *et al.* (19, 20) and STUTZ and WATANABE (22) is correct.

There is, however, a fact that the dwarf-1 corn seedling can be induced to elongate by gibberellin but not by indole derivatives including IAA and others (17). BRIAN and HEMMING (3) have shown that the auxin applied exogenously does not accelerate the growth of dwarf peas appreciably. And there is no evidence that the auxin supply limits the growth of dwarf peas (1). CURTIS (6) observed that GA, but not IAA, reverses the inhibition of growth of bean plant (var. Black Valentine) induced by the culture filtrate from *Aspergillus niger*. Moreover, the senior author observed that the shoot inhibition induced by high concentration of auxins was reversed by high concentrations of gibberellin (11). MALTZAHN and MACQUARRIE (14) reported that the growth of protonemata of *Splachnum ampullaceum* (L.) HEDW. was promoted by GA but not by IAA, vitamins and amino acids. NITSCH (16) reported that GA-treatment of some woody plants increased their auxin content but recently RICARD and NITSCH (21) found the fact that, in very young tissues of wheat coleoptile, IAA was not a natural growth promoter but at least two acidic substances other than IAA were responsible for the growth. They also

demonstrated that kinetin and gibberellin had a promoting effect on this stage of coleoptile. APPLEGATE (2) observed, using seedlings of *Zinnia elegans* (var. Scarlet Flame), no difference between the effect of GA and that of GA mixed with TIBA in various concentrations, and concluded that auxin was not involved in the GA-induced cell elongation. These results do not support the hypothesis presented by PILET *et al.* (19, 20) and STUTZ and WATANABE (22). The hypothesis that gibberellin action is due to a change in auxin content in plant tissues has to be revised.

Summary

Gibberellic acid and gibberellin A did not significantly affect the activity *in vitro* of indoleacetic acid oxidase prepared from etiolated Usui pea seedlings. This was confirmed at pH's between 3.0 and 8.0.

Activity of the enzyme prepared from seedlings pretreated with gibberellic acid did not differ from that prepared from untreated ones.

At high concentrations gibberellic acid inhibits the color development of Salkowski reaction more strongly than gibberellin A.

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