STIMULATION OF COLEOPTILE ELONGATION IN ZEA MAYS BY p-HYDROXYBENZOIC ACID

by

John T. Romeo and Karl Folkers*

Institute for Biomedical Research
The University of Texas at Austin
Austin, Texas 78712

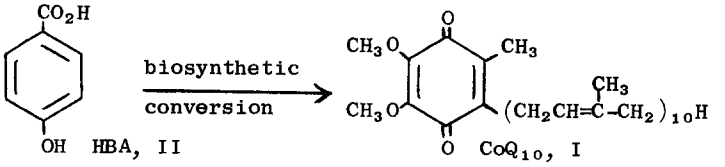
Received January 15,1975

SUMMARY

Based on the biochemistry of coenzyme Q and plastoquinone, corn coleoptile sections were treated with p-hydroxybenzoic acid (HBA) and p-hydroxyphenylpyruvic acid (HPPA), which are biosynthetic precursors of the essential coenzyme Q_{10} and plastoquinone-9, respectively. HBA at low concentrations stimulated growth; higher concentrations inhibited elongation. HPPA did not stimulate growth, but inhibited growth. HBA could promote growth by benefiting respiration particularly if a deficiency of HBA existed and there were a depressed biosynthesis of coenzyme Q_{10} for electron transfer in respiration.

INTRODUCTION

Coenzyme Q_{10} (CoQ_{10}, I) is found in the mitochondria of cells, and acts as an oxidation-reduction carrier in the electron transport system which catalyzes the oxidation of succinate and NADH by molecular oxygen. Friis et al. (1) worked out the complete biosynthesis of CoQ_{10} from p-hydroxybenzoic acid (HBA, II); this acid is the key biosynthetic precursor in mammalian cells and cells of higher plants. The distribution of CoQ_{10} has been summarized (2).



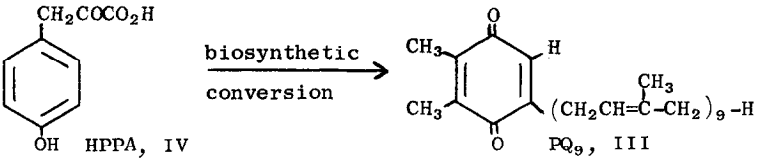
p-Hydroxybenzoic acid has been found in 97% of 122 plant species which were analyzed (3). Vieitez et al. (4) isolated HBA from Ribes rubrum and showed it to be a growth promoter when applied at low concentrations (20-100 μg/ml) to Avena coleoptiles. Pilet (5) observed growth promoting effects on the stems of Lens culinaris using synthetic HBA. Kowes (6) and Vieitez et al. (4) inhibited Avena coleoptile elongation with higher concentrations of HBA. Van Sumere et al. (7) observed some increase in the germination of barley and lettuce seed with low concentrations of HBA, and at higher concentrations, they observed that germination was inhibited.

Plastoquinone (PQ_9, III), found in the chloroplasts of higher plants, plays

*Coenzyme Q. 185.

a role in photosynthesis analogous to that of coenzyme Q_{10} in mitochondrial respiration (8). It acts as an oxidation-reduction carrier mediating electron transfer between chlorophyll and the cytochromes which lead to the reduction of NADP (9). Plastoquinone occurs widely in chlorophyll containing tissue and is absent from most non-photosynthetic tissue. It is present in all those algae and higher plants which use water as a source of reducing power (10).

Whitstance and Threlfall (11) have shown that in maize shoots p-hydroxyphenylpyruvic acid (HPPA, IV) is biosynthetically incorporated into plastoquinone via homogentisic acid.



The present experiments were designed to extend the knowledge on HBA as a plant growth regulator, to observe its effects on the economically important Zea mays. Also, HPPA, the apparent analog of HBA, in the photosynthetic process, was studied for plant regulatory effects.

EXPERIMENTAL

Seeds of Zea mays (corn) were soaked 24 hours in distilled water, sown on wet filter paper in Petrie dishes and allowed to germinate and grow in darkness at 72° F for 144-168 hours. Seedlings of uniform ages and lengths were selected and the procedure of Muir and Hansch (12) for excising 2 mm coleoptile sections was used. The problem of obtaining sections of uniform lengths was resolved by the following procedure. Two pieces of heavy tape were fixed 2 mm apart on a Petrie dish. Seedling tips were placed against the edge of one tape, and the section cut made along the edge of the other tape with a razor blade. With a little experience one obtains sections of uniform lengths varying by not more than 0.1 mm.

Sections were measured to the nearest 0.03 mm with an ocular micrometer mounted on a binocular dissecting scope. Sections were placed in Petrie dishes (5/dish) on filter paper moistened with 5 ml of varying concentrations of HBA or HPPA solutions (10^{-2} - 10^{-8} M) or a combination of the two. A water control was run with each series. The dishes were placed in darkness and growth was measured after 24 and 48 hours.

RESULTS

Coleoptile elongation is expressed as percent change from growth of control in Tables 1, 2 and 3. Each percentage figure is an average of the elongations of 5 sections in a series. The average percent elongation for all series is also given.

Table 1. COLEOPTILE GROWTH WITH VARYING CONCENTRATIONS OF HBA. EXPRESSED AS PERCENT CHANGE FROM CONTROL.

	HBA Molar Concentration						
Series	-8	-7	-6	-5	-4	-3	-2
A	+12.6	-3.2	-1.1	+2.1	-13.7	-13.7	-92.6
B	+13.4	+26.0	+24.4	+3.4	-3.4	+7.6	-84.1
C	-10.5	+33.3	-6.1	+5.3	-9.6	+17.5	-93.0
D	+10.0	+2.8	-2.8	-16.1	+2.8	+12.2	-92.8
E	+3.5	0	+33.7	+25.6	+41.9	+10.5	
F	+30.5	+10.2	-1.7	+11.9	-1.7	+1.7	
G	+4.1	-4.1	+24.3	+20.3	+6.1	+16.2	
H	-3.4	+12.3	+4.4	+9.8	-12.3	+0.5	
I	0	+10.2	+13.0	-26.5	+14.9	-6.5	
J	-4.1	+9.2	+10.7	-10.2	-7.1	-8.2	
K	-15.0	+5.5	+13.4	+21.2	+14.2	+15.0	
L	-23.2	+11.2	+9.7	+7.5	-35.8	-40.0	-75.4
Average	+1.5	+9.4	+10.2	+4.5	-0.3	+1.1	-87.6

Table 2. COLEOPTILE GROWTH WITH VARYING CONCENTRATIONS OF HPPA. EXPRESSED AS PERCENT CHANGE FROM CONTROL.

	HPPA Molar Concentration						
Series	-8	-7	-6	-5	-4	-3	-2
A	-1.2	-18.1	-2.4	-4.8	-25.3	-6.0	
B	-5.9	-1.0	+11.2	-7.8	-12.8	+6.9	
C	+5.6	+15.7	-9.0	+6.7	+30.3	+22.5	
D	-8.8	+4.7	-6.1	+8.1	-12.8	+11.5	
E	-3.7	+13.9	-23.2	-23.2	-26.9	-3.7	
F	0	+12.8	-22.9	-20.0	-8.6	+5.7	-67.2
G	-1.4	+24.3	+10.0	+2.9	+10.0	-18.6	-7.9
H	+17.7	+11.4	+3.8	+15.2	+22.8	+1.3	
I	-2.8	-11.9	-10.1	-15.6	-9.2	-17.4	
Average	-0.2	+5.7	-5.4	-4.3	-3.6	+0.2	-37.5

Significant stimulation of growth occurred with concentrations of HBA in the 10^{-7} - 10^{-5} molar range. The t test of statistical significance for paired comparison is significant at the 2% level for the 10^{-6} M concentration. Significant inhibition was observed only with the 10^{-2} molar solution. The maximum stimulation at 10^{-6} M compares favorably with the data of Vieitez et al. (4), who obtained maximum stimulation of Avena coleoptiles at 50 μg/ml, and of Pilet (5), who observed maximum stimulation of Lens stems at 10^{-6} M HBA. The concentration at which inhibition first occurs in corn coleoptiles is higher than that for Avena coleoptiles or Lens stems.

Table 3. COLEOPTILE GROWTH WITH VARYING CONCENTRATIONS OF HBA + HPPA. EXPRESSED AS PERCENT CHANGE FROM CONTROL.

	HBA + HPPA Molar Concentration						
Series	-8	-7	-6	-5	-4	-3	-2
A	+9.5	+33.3	+20.6	+41.3	-1.6	+9.5	
B	+7.0	+2.8	+25.4	+16.9	-8.5	+9.9	
C	+3.6	+17.1	+6.1	-8.5	-2.4	-8.5	
D	+7.6	-15.2	+12.6	+16.4	+15.2	+12.6	
E	-5.7	-5.7	+17.1	-8.6	-1.4	-8.6	
F	+2.2	-7.5	+1.1	-17.2	-12.9	-4.3	
G	-11.9	+1.0	0	+5.0	-2.0	-12.9	-96.0
H	+18.8	+34.4	+26.0	+5.2	-14.6	-17.7	-100.0
Average	+3.9	+7.5	+13.6	+6.3	-3.5	-2.5	-98.0

p-Hydroxyphenylpyruvic acid did not appear to affect coleoptile elongation under the experimental conditions which were used, but did show a strong inhibition at a concentration of 10^{-2} molar. HPPA when applied together with HBA had no synergistic or inhibitory effect. The growth curve was similar to that obtained with HBA alone.

DISCUSSION

The data in Tables 1 and 3 show the variations among series in the amount of growth stimulation at a given HBA concentration. These variations in growth may be partially explained by the differences in ages of the different series (varying from 144-168 hours), and the slightly differing lengths of the original seedlings from which coleoptile sections were made. Errors of technique were minimized with experience, and the variations reported herein are believed to be due to causes inherent in the individual plants.

To explain the varying effects of concentration of HBA on growth, several hypotheses have been proposed, but none of these hypotheses took into account the present knowledge on the role of HBA as a precursor of CoQ_{10}. Zenk and Muller (13) and Tomaszewski (14) showed that HBA at 10^{-4} M concentration increases IAA decarboxylation in Avena coleoptile tissue and inhibited growth. Tomaszewski (3) suggested that the interaction of HBA with endogenous auxin to form phenol-auxin complexes might cause elongation of plant sections. Pilet (5) demonstrated that HBA at all concentrations acted as an activator of the IAA oxidizing system and produced a decrease in the endogenous level of auxin which can be extracted from Lens stems. Van Sumere et al. (7) hypothesized that HBA and other "phenolics" inhibit the transfer of amino acids and the formation of proteins necessary for germination in seeds. They found that all

of the substances which inhibit or activate yeast growth are also active in lettuce and barley seeds.

While auxin decarboxylation or inhibition of amino acid transfer may possibly explain inhibitory growth-germination effects with high HBA concentration, we believe that other biochemical processes concerned with growth must be operating when stimulatory effects are observed with the lower concentrations. Vieitez et al. (4) obtained greater growth stimulation of Avena coleoptiles when HBA at low concentrations was added with IAA than with IAA alone.

On the basis of the known function of CoQ_{10} and its biosynthesis from HBA, it is reasonable to believe that HBA may be affecting growth phenomena by directly affecting respiration, particularly if a deficiency of HBA existed and there were a depressed biosynthesis of CoQ_{10} for respiration. In support of this interpretation, Van Sumere et al. (7) provided evidence that both yeast respiration and respiration of germinating lettuce and barley seeds were increased at 10^{-3} M concentration HBA.

It seems likely then that at lower concentrations of HBA, where auxin decarboxylation is less, that an increase in growth-germination phenomena and growth of coleoptiles could be caused by promotion of bioenergetics due to increased respiration resulting from increased biosynthesis of the vitamin, coenzyme Q_{10}.

Coenzyme Q_{10} showed vitamin activity in several syndromes of animal and human nutritional and genetic diseases (15). Corresponding nutritional deficiency states caused by soil factors, disease, and environmental stress, or genetic defects caused by excessive inbreeding, use of pesticides and environmental stress may produce coenzyme Q_{10} deficient plants. Such a deficiency might be relieved by treatment with HBA. This explanation accounts not only for the observed growth stimulatory effects of HBA, but also for the variations in response among individual plants. The effect could be expected to depend on an individual plant's need; those with the greater deficiencies presumably yielding greater positive responses.

The lack of growth stimulation by p-hydroxyphenylpyruvic acid may be merely a failure to find the appropriate experimental conditions. Lower concentrations and growth tests performed in light rather than in darkness might be appropriate.

REFERENCES

1. Friis, P., Daves, G.D., and Folkers, K., J. Am. Chem. Soc. 88, 4754 (1966).
2. Crane, F.L., Biochemistry of Quinones, R.A. Morton, ed. Acad. Press, London, New York, 183 (1965).
3. Tomaszewski, M., Regulateurs Nat. Croissance Vegetale, Paris, 335-351 (1964).
4. Vietez, E., Seoane, E., Gesto, D.V., Mato, C., Vazquez, A., and Carnicer, A., Phys. Plantarum., 19, 294-307(1966).

5. Pilet, P.E., Phytochem., 6, 77-82 (1966).
6. Kowes, E., Acta. Biol. Szeged. 3, 179-187(1957).
7. Van Sumere, C.F., Cottenie, J., De Greef, J., and Kint, J., Recent Advances in Phytochemistry, 4, Appelton Century Crofts, 165-221 (1972).
8. Crane, F.L., Plant Physiol., 34, 128 (1959).
9. Butler, W.L., Accts. of Chem. Res., 6, 177-184 (1973).
10. Redfearn, E.R., Biochemistry of Quinones, R.A. Morton, ed. Acad. Press, London, New York, 158 (1965).
11. Whistance, G.R. and Threlfall, D.R., Biochem. J., 117, 593-600 (1970).
12. Muir, R.M., and Hansch, C., Plant Physiol., 28, 218-231 (1953).
13. Zenk, M.H., and Muller, G., Nature, 200, 761-763 (1963).
14. Tomaszewski, M., Bull. Acad. Polon. Sci. Ser. Sci. Biol., 8, 61-63 (1960).
15. Folkers, K., Inter. J. Vit. Res., 39, 334 (1969).