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Control of Growth by Auxin and Its Specific Neutral Inhibitor

R. M. MUIR

Department of Botany, University of Iowa
Iowa City, Iowa, U.S.A.

Abstract. Evidence for the existence of a neutral inhibitor specific for auxin has been in the literature for 45 years. The inhibitor is demonstrable by its effect in causing positive curvature of the *Avena* coleoptile. The growth of the mesocotyl of oat and corn seedlings in darkness and its inhibition by light are determined by the neutral inhibitor as is the phototropic response of the sunflower stem. Production of the inhibitor is promoted by red and fluorescent light. Irradiance at 730 nm promotes auxin production while irradiance at 660 nm promotes production of the inhibitor. The positive curvature induced by 2,3,5-triiodobenzoic acid can be used to quantify the neutral inhibitor. Since the benzoic acid is more effective than iodoacetate in reacting with the sulfhydryl of cysteine, a sulfhydryl group is indicated to be one reaction site for auxin and to be the basis of polar transport.

Two aspects of plant growth that have been investigated extensively without satisfactory resolution are the growth of the mesocotyl of grasses and the phototropic response of plants to light. The measurements of auxin in the control of growth have employed the *Avena* curvature bioassay for auxin in the diffusates from plant tissues with the assumption that only a promotive substance is present when, in fact, an inhibitor specific for indol-3-ylacetic acid (IAA) is also present. Such an unknown inhibitor would obscure the results of other methods of determining IAA content and distribution as well.

GROWTH OF THE MESOCOTYL

WENT (1928) proposed that the growth of the mesocotyl is limited by the amount of growth hormone that is received from the apical portion of the coleoptile. VAN OVERBEEK (1936) from his experiments also concluded that the reduction of mesocotyl growth is due to a decreased amount of auxin produced by the tip of the coleoptile of the corn seedling. More recently the investigations of IINO and CARR (1982) have caused them to conclude that the growth of the apical growing region of the mesocotyl depends on the amount of IAA from the coleoptile tip.

Opposed to this view of the control of mesocotyl growth by the coleoptile is the finding by SCHNEIDER (1941) that the effect of light in inhibiting the growth of the mesocotyl is a direct response of the mesocotyl and not depen-

dent on the coleoptile. The independence of mesocotyl growth from auxin production in the coleoptile tip was also demonstrated in the difficult experiments of MER (1951), performed in darkness, in which the removal of the coleoptile tip did not affect the growth of the mesocotyl of *Avena* seedlings. However, IINO and CARR (1982) have reported that removal of the coleoptile tip of *Zea* seedlings under infra-red radiation caused a slight reduction of mesocotyl growth in their experiment.

Earlier measurements of diffusible auxin in coleoptiles have shown decreasing amounts of auxin below the coleoptile tip. If the diffusates are collected in agar blocks buffered at pH 3.5, the amount of auxin collected at the base of the entire coleoptile is the same as that collected from the tip in *Avena* seedlings grown in darkness for four days, and the amount collected from the entire coleoptile and mesocotyl of similar *Zea* seedlings is greater than the amount from the coleoptile tip (KESSLER 1981). After five days in darkness the mesocotyl of the *Avena* seedling ceases growth even though more than half of the maximum level of auxin is present at the base of the coleoptile. At this time positive curvatures of the test coleoptiles are obtained with agar blocks containing the diffusates from the mesocotyl indicating that the auxin content is counteracted by an inhibitor present in the diffusate. When *Zea* seedlings are exposed to red light 12 hours before the measurement of diffusible auxin, the growth of the mesocotyl is reduced and positive curvatures of the test coleoptiles are induced by the agar blocks containing the diffusates.

Positive curvatures of the *Avena* coleoptile caused by extracts and diffusates from plant tissue have been reported several times in the literature (SNOW 1939, STEWART *et al.* 1939, SHIBAOKA 1961, BRUINSMA *et al.* 1975). Whereas the negative curvature of the coleoptile in the bioassay represents a preponderance of a promotive substance in the agar block on the growth of the tissue which has been depleted of endogenous hormone by prior removal of the tip, the positive curvature results from lesser growth on the side to which the block is applied and represents a preponderance of an inhibitory substance in the block on the endogenous hormone that is present in the tissue. For curvature to occur the inhibitor must also have the unique property of the hormone, polar transport.

The standard *Avena* curvature bioassay for auxin which employs two successive decapitations of the coleoptile to deplete the endogenous hormone and reduce the growth of the coleoptile is obviously not suitable for the measurement of an inhibitor of auxin-induced growth. After one decapitation a new site of synthesis of auxin is regenerated and 6 hours later the growth rate of the coleoptile is about 65 % of that of an intact coleoptile (VAN OVERBEEK 1941). With *Avena* seedlings treated in this way the bioassay of the inhibitor becomes possible (KESSLER 1981). If the diffusate from the plant tissue is collected in agar blocks buffered at pH 8.5, the IAA in the diffusate is almost totally dissociated and does not enter the coleoptile tissue so that the maximum effect of the inhibitor is obtained. Since 2,3, 5-triiodobenzoic acid (TIBA) was found to reduce the effect of IAA in causing negative curvature of coleoptiles (FERGUSON 1971), it was examined for an effect in positive curvature and gave curvature that was proportional to the logarithm of the concentration at 10^{-7}M to 5×10^{-5} (KESSLER 1981). Thus it is possible to relate the amount of the inhibitor to quantities of TIBA causing the same amount of positive curvature.

The positive curvature bioassay for the inhibitor of auxin-induced growth has been used to determine the relationship of the inhibitor and the growth of the *Avena* mesocotyl in darkness. The inhibitor is detected in the agar blocks receiving the diffusate from two mesocotyls at the third day of growth and increases to a maximum on the fourth day after which it diminishes (KESSLER 1981). The mesocotyl increases in length until the fifth day and then ceases growth even though a considerable quantity of auxin is present at the base of the coleoptile. It is evident that the control of growth in the mesocotyl is not dependent on auxin from the coleoptile but is determined by the inhibitor formed during its development.

PHOTOTROPIC GROWTH

WENT (1928) proposed that the growth response due to unilateral light was the result of lateral movement of the growth hormone to the shaded side of the tissue causing greater growth on that side and curvature toward the source of light. Alternative explanations have involved light destruction of auxin or of the enzyme system forming auxin but studies of the phototropic response of the sunflower plant have implicated an inhibitor of auxin-induced growth rather than an unequal lateral distribution of auxin. SHIBAOKA (1961) discovered a neutral inhibitor in extracts and diffusates from sunflower leaves which suppressed IAA-induced *Avena* curvature and epicotyl growth of the sunflower. A neutral inhibitor also was obtained by LAM and LEOPOLD (1966) from sunflower stems in their experiments on the effects of light on curvatures of the hypocotyl. BRUINSMA *et al.* (1975) did not find an effect of unilateral light on the lateral distribution of endogenous IAA in sunflower hypocotyls and suggested that it is an inhibitor that is unevenly distributed due to unilateral light.

Sunflower plants grown in light and similar to those employed by LAM and LEOPOLD have been examined for auxin and the inhibitor in diffusates by means of the negative curvature and positive curvature of the *Avena* coleoptile (MUIR and ZHU 1983). Diffusates were collected at the base of the hypocotyl for plants that had the apex and one cotyledon removed before being given various light treatments. Both auxin and its inhibitor were present in diffusates from plants placed in darkness for 4 and 3 hours. The inhibitor content of diffusates from plants under fluorescent light or irradiance at 660 nm was increased over the amount in the diffusates from plants in darkness. The auxin content of the diffusates from plants given irradiance at 730 nm was increased. Similar results were obtained for diffusates from the hypocotyl and a leaf of plants that had the apex and cotyledons removed before being given the light treatments and for diffusates from the half-expanded leaves of intact plants 21 days old.

The auxin of the sunflower plant is IAA but the identity of the inhibitor is unknown. SHIBAOKA (1961), LAM and LEOPOLD (1966) and THOMPSON and BRUINSMA (1977) all agree that the inhibitor is a neutral substance and the positive curvature bioassay is based on the non-acidic nature of the compound. Xanthoxin has been identified as an inhibitor in the sunflower seedling (THOMPSON and BRUINSMA 1977, FRANSSEN and BRUINSMA 1981) but it does not cause positive curvature of the *Avena* coleoptile.

MECHANISM OF ACTION

At a concentration of 7×10^{-6} M, TIBA is a competitive inhibitor of the negative curvature of *Avena* coleoptiles induced by IAA, and IAA at a concentration of 1.55×10^{-7} M is a competitive inhibitor of the positive curvature induced by TIBA. This evidence that the molecules are acting at a common site corresponds with the auxin activity of TIBA in promoting the straight growth of coleoptile segments (MUIR and HANSCH 1961). The TIBA also apparently has the optimal value for the approximate superdelocalizability at the ortho position for a reaction with a nucleophilic reagent (−SH) as calculated by FUKUI *et al.* (1958) for a series of substituted benzoic acids.

The observation by LEOPOLD and PRICE (1956) that TIBA is ten-fold more reactive with cysteine than is iodoacetate indicates a specific activity of TIBA for the sulfhydryl group and would involve the displacement of the iodine at the ortho position forming a thio-ether bond at the reaction site as demonstrated for the reaction of iodoacetate with cysteine by DICKENS (1933). These findings corroborate the hypothesis of a two-point reaction of the auxin molecule involving the carboxyl group and a position on the aromatic ring (MUIR and HANSCH 1951). Furthermore, polarity of transport appears to be a matter of sulfhydryl gradient in the tissue. These conclusions are the same as those of Shibaoka 23 years ago.

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BOOK REVIEW

BRIGGS, W. R., JONES, R. L., WALBOT, V. (ed.): ANNUAL REVIEW OF PLANT PHYSIOLOGY. VOL. 35, 1984. — Annual Reviews Inc., Palo Alto, Cal., U.S.A. 736 pp., U.S. $ 27.00 (U.S.A.), 30.00 (elsewhere).

The yearly volumes containing review papers in various fields of plant physiology belong to the most popular literature at universities and in research institutes all-over the world. The rate of citation of these reviews is extremely high and thus they control the level of knowledge in plant physiology. Of course, the responsibility of their authors is high: any error in the text may be introduced into science as a respected fact.

The 35th volume brings 22 reviews written by 29 authors from the U.S.A. (17 authors), the F.R.G. (5), Australia (4), Israel (2), and Japan (1). In addition, a prefatory chapter was prepared this time by C.R. Stocking of the University of California. It deals with the life experience of this outstanding plant physiologist in the field of research (chloroplast-cytoplasmic interactions, food chemistry), research planning (on duplication of projects), publishing, and developing a botany department, as well as his thoughts on the past and future research in photosynthesis.

According to the Editors of the volume, the reviews may be divided according to the level of organization of the objects. The range "Molecules and Metabolism" contains eight reviews: on immunoassays of plant growth regulators, on structure, synthesis, function, and regulation of chloroplast ATPase, excess sulphur, ethylene, and seed proteins (four reviews), on leghemoglobin and *Rhizobium* respiration, and on photorespiration and photosynthetic electron transport pathways and mechanisms (two reviews). The range "Organelles and Cells" contains the topics: transport of sugars, amino acids and further compounds (two reviews), biogenesis of glyoxysomes, use of NMR spectroscopy for metabolic studies, and expansion of plant cells. Five reviews in the range "Tissues, Organs, and Whole Plants" deal with: root development regulation, role of phytoalexins and their elicitors in defense against microbes, osmoregulation and water stress, cell division patterns, and quantitative description of development. The last range "Population and Environment" contains four topics: photoinhibition of photosynthesis, crown galls, isolation and characterization of mutants in cell cultures, and freezing injury and cold acclimation.

The sequence of reviews in the volume is different: I was not able to find the logic. Of course, all the mentioned topics also contain related secondary problems, *e.g.*, photoinhibition of photosynthesis also includes the interaction of light and (high or low) temperature stresses or herbicide and water stresses; water relations of plants are also dealt with in reviews on the description of development, isolation of mutants, freezing injury, plant cell expansion, and photoinhibition of photosynthesis.

The reviews are traditionally accompanied by no or only a few figures — in this volume the most richly illustrated (10 figures) is the review on quantitative descriptions of development. I do not know what the reason is — to spare space, the copyright problems? In some of the present reviews figures would help in understanding the rather complicated text. The supplemented Author Index shows the places in the text where the author is cited only by a number: addition of this number in italics in the Index would much help in its use. The Subject Index is very good.

In general, the reviewed volume belongs to those which must be in every plant physiological library.

Z. ŠESTÁK (*Praha*)