Sequence of Key Events in Shoot Gravitropism

Received for publication August 29, 1983 and in revised form January 4, 1984

FERNANDO MIGLIACCIO and DAVID L. RAYLE*
Department of Botany, San Diego State University, San Diego, California 92182

ABSTRACT

It has recently been shown that asymmetric acid efflux is closely correlated with the gravitropic curvature of plant shoots and roots. The research reported here addresses whether auxin (IAA) redistribution in shoots is the cause or result of asymmetric acid efflux.

When abraded sunflower (Helianthus annuus cv Mammoth) hypocotyls are submerged in 20 millimolar neutral buffer, gravicurvature is greatly retarded relative to 0.2 millimolar controls. Nevertheless, in both buffer systems there is a similar redistribution of [3H]IAA toward the lower surface of gravistimulated sunflower hypocotyls. These results suggest that graviperception initiates IAA redistribution, which in turn results in auxin-induced asymmetric H⁺ efflux across the shoot. This interpretation is reinforced by data showing the effects of removal of the epidermal layers (peeling), osmotic shock, and morphactin treatment on gravicurvature and [3H]IAA redistribution. Peeling and osmotic shock inhibit gravicurvature but not redistribution. Morphactin inhibits both processes but does not inhibit hypocotyl straight growth.

Gravitropically stimulated roots and shoots exhibit asymmetric proton release across their growing axis. In shoots the lowermost tissues exhibit enhanced acid release, while in roots the gradient is reversed (14–16, 23, 24). Evidence that such asymmetric acid release initiates asymmetric cell elongation (i.e. acid growth) and thus gravitropism derives from three kinds of observations. First, asymmetric application of acid solutions causes asymmetric cell elongation and curvature (1, 9, 18, 24). Second, inhibition of cellular proton extrusion prevents gravicurvature (14, 24). Last, infiltration of shoot tissue with neutral buffers inhibits gravitropism (23).

As auxin is well known to stimulate proton extrusion from coleoptile, hypocotyl, and epicotyl sections (2, 12, 13, 17), one might imagine auxin redistribution in gravitropically stimulated shoots is responsible for initiating asymmetric acid efflux. This is essentially a restatement of the classic hypothesis of Cholodny and Went (22), modified to include the capacity of auxin to stimulate cellular proton extrusion. Alternatively, one might speculate that gravity perception mechanisms directly initiate asymmetric proton release and auxin redistribution occurs secondarily in response to this gradient. This latter possibility would be consistent with the tenets of the chemiosmotic theory of auxin transport (6, 7). This paper reports experiments designed to test these two alternatives.

MATERIALS AND METHODS

Plant Material. Seeds of Helianthus annuus cv Mammoth were soaked in tap water for 30 min, sown on wet vermiculite, and germinated in climatic chambers programmed for cycles of 16 h of light (500 μE m⁻² s⁻¹ at 25°C) and 8 h dark (20°C). After 5 d growth, 4-cm long hypocotyl sections were isolated from the region beginning immediately below the cotyledonary node. In most experiments, hypocotyl sections were abraded by stroking (five strokes/segment) with a paste prepared by mixing 1 part water with 2 parts rottenstone (finely decomposed siliceous limestone; Donovan Products Inc., Long Beach, CA).

Determination of [3H]IAA Redistribution and Magnitude of Gravicurvature. After preparation hypocotyl segments were placed upright (to avoid gravistimulation) in a vial containing 0.2 mM phosphate buffer pH 7.0 and [3H]IAA at 10⁻⁸ M (Research Products International Corp.; specific activity, 29 μCi/μmol). After 1 h, the sections were rinsed rapidly with distilled H₂O and mounted horizontally in custom Plexiglas holders (23, 24). To prevent rotation or movement, the basal part of each hypocotyl was embedded in liquid 3% agar which quickly solidified. Initially about 2.5 cm of each shoot segment extended past the edge of the holder. In most experiments the holders were then submerged in breakers containing 1000 ml of test solution maintained at 30°C by a thermostatic bath. All manipulations prior to submerging the holders were carried out under normal laboratory lighting, while gravistimulation and curvature occurred in darkness. In one experiment (the kinetics of H redistribution) the holders were placed in a humidified chamber (30°C; saturated by continual misting) rather than submerged in solution. Determination of the magnitude of gravicurvature was accomplished as previously described (23, 24).

Following variable periods of gravistimulation, hypocotyls were marked with ink dots to facilitate subsequent recognition of their upper and lower surfaces during the period of gravistimulation and then removed from the holders. In some experiments the segments were cut along their longitudinal axis to yield upper and lower halves corresponding to their relative positions during the period of gravistimulation. In other experiments a similar procedure was followed except that the upper and lower epidermis was stripped with forceps under a stereoscopic microscope. After weighing the halves sections and/or epidermal strips, radioactivity was determined by liquid scintillation counting. After normalizing for fresh weight, the per cent cpm in the lower part of the hypocotyl relative to the cpm in the upper part was calculated. Henceforth, we will refer to these values as per cent redistribution.

In two sets of experiments, the above procedure was modified slightly. In those involving osmotic shock, hypocotyls were subjected to a solution of mannitol at −1.21 mPa of osmotic potential for 30 min and then 3 min washing in 0.5 mM CaSO₄ solution prior to isotope uptake. In another set of experiments, the epidermis was completely stripped prior to isotope uptake.

Chromatographic Analysis of Extracts. After [3H]IAA uptake and subsequent gravistimulation (3 h), hypocotyl segments were cut along their longitudinal axis to yield upper and lower halves as described above. Half sections (24/treatment) were then extracted overnight in 4 ml of methanol (0°C). To allow later corrections for concentration and dilution, we added 2 μl of a

\[^{1}\text{Supported by National Aeronautics and Space Administration Grant NA6W-230.}\]
solution of [2-14C]IAA (New England Nuclear) (a total of 79,000 dpm) to each methanolic sample. The volume of each sample was reduced to 200 to 250 µl in a vacuum desiccator. A 50-µl aliquot was subjected to HPLC in a Varian model 5000 as described by Hollenberg et al. (8). The column (C18 reverse phase) was a Varian MCH-10, 30 cm x 4 mm. Eluting solvents A (1% glacial acetic acid) and B (acetonitrile) were supplied as follows: pure A from 0 to 15 min, a linear gradient from 0 to 30% B from 15 to 30 min, and a linear gradient to 100% B from 30 to 45 min. The flow rate was 1 ml/min. Fractions (20 s) were collected and each was added to 10 ml of Bray’s solution for counting in a Beckman Series 9000 liquid scintillation counter with a data-reduction module that calculated dpm. Using total 14C counts eluted, 3H elution data were corrected for concentration and dilution, and then plotted. 14C counts were also used as an internal standard. In this system IAA was eluted after 36 min.

**RESULTS AND DISCUSSION**

We previously reported that high molarity (e.g. 20 mM) neutral buffers inhibit shoot gravitropism by preventing the establishment of an acid asymmetry across the shoot axis (18, 23, 24). To determine whether the lateral transport of auxin is also inhibited under such conditions the distribution of [3H]IAA was followed in gravstimulated sunflower hypocotyls submerged in either 0.2 or 20 mM K-phosphate at pH 7. Shoots submerged in either high or low molarity buffer exhibit a similar redistribution of radioactivity toward their lower surfaces although only the low molarity-treated shoots displayed substantial curvature (Fig. 1; 23).

To determine if the redistribution of label shown in Figure 1 represented a lateral transport of applied [3H]IAA (rather than IAA metabolites or IAA conjugates) segments were treated as described above and the extractable radioactivity subjected to chromatographic analysis. The bulk of the extracted radioactivity is attributable to IAA and no appreciable changes in metabolism appear to have resulted from the buffer treatments (Fig. 2).

The data presented in Figures 1 and 2 suggest that graviper-

---

**Fig. 1.** 3H Redistribution in gravstimulated sunflower hypocotyls incubated in 0.2 or 20 mM phosphate buffer (pH 7.0). Data are expressed as % increase in cpm in the lower half of the hypocotyls relative to the upper half. Bars represent se; n = 7.

**Fig. 2.** HPLC elution profiles of methanolic extracts of sunflower half sections. Sections were incubated in either 0.2 mM (A and B) or 20 mM (C and D) phosphate buffer (pH 7.0) prior to bisection and analysis. The peak at 36 min corresponds to the retention time for IAA standards.

**Fig. 3.** Kinetics of [3H]IAA redistribution in sunflower hypocotyls incubated in humid air: epidermal strips (●), hypocotyl half sections (▲). Bars represent se; n = 4.

**Fig. 4.** 3H redistribution and gravicurvature in peeled hypocotyls incubated in 0.2 mM phosphate buffer (pH 7.0). Curvature data (open bar, peeled hypocotyls; shaded bar, controls) were obtained after 3 h of gravstimulation. Bars represent se; n = 7.
The latter which responsible possibility exception mechanisms rather than an acid gradient are directly responsible for auxin redistribution. Further, these data raise the possibility that auxin redistribution precedes and initiates the asymmetric acid release which results in asymmetric cell extension. The following experiments were designed to test this possible sequence of events.

The kinetics of [3H]IAA redistribution in gravitropically stimulated hypocotyl segments are detailed in Figure 3. As shown an asymmetry is present after 15 to 20 min. In our hands, there is a 20- to 30-min lag period before such sections exhibit gravicurvature. Thus, these data are consistent with a causal relationship between auxin redistribution and curvature. Note that the magnitude of IAA redistribution is accentuated in epidermal peels relative to half sections. A similar phenomenon has been reported by Iwami and Masuda (10, 11). Our data differ quantitatively, however, from the data reported by Iwami and Masuda (10, 11). The latter authors report upper versus lower redistribution ratios approaching 1:4 after several hours. We find ratios closer to 2:3 which is similar to those reported by Gillespie and Thimmann (5).

The difference noted between halves and epidermal peels prompted us to look more closely at the role of the epidermis in auxin redistribution and asymmetric growth. As shown in Figure 4, sunflower hypocotyls stripped of their epidermal layer respond very poorly to gravitropism. This result confirms previous reports by Firn and Digby (3, 4) and Iwami and Masuda (10, 11). Nevertheless, gravistimulated peeled sections are capable of limited auxin redistribution (Fig. 4). We believe these data are best rationalized by assuming that the majority of the cells within the hypocotyl participate in auxin redistribution; however, only the epidermal layers are capable of rapidly responding to auxin. Auxin-induced straight growth is also inhibited by removal of the epidermal layers (3, 13).

Further evidence consistent with the notion that the redistribution of IAA within gravitropically stimulated hypocotyls is not driven by asymmetric acid efflux derives from experiments utilizing osmotic shock. Rubinstein and co-workers (19, 20) have shown that osmotic shock causes a transient inhibition of IAA and fuscoacin-induced H^+ secretion and, thus, one might predict such treatment would also retard gravicurvature. This was indeed found to be the case. Shocked hypocotyls exhibit a 2- to 3-h lag before curvature commences (data not shown). Auxin redistribution, however, is not affected by osmotic shock (Fig. 5).

Last, we investigated the effect of morphactin on [3H]IAA redistribution and gravitropism. In a variety of systems, morphactin has been reported to be a powerful inhibitor of auxin transport and tropisms but not straight growth (21 and references therein). As shown in Figure 6, morphactin treatment prevents the lateral redistribution of applied [3H]IAA. Consistent with the notion that auxin redistribution is required for asymmetric growth, morphactin strongly inhibits sunflower gravicurvature (Fig. 6).

We believe the experiments reported herein provide strong evidence that auxin redistribution in sunflower hypocotyls is directly initiated by graviperception mechanisms and it is not a secondary event initiated by asymmetric growth or acid efflux. Further, we believe these experiments indicate the likely sequence of events in shoot gravitropism is as follows: graviperception, auxin redistribution, auxin-mediated asymmetric acid efflux and, last, asymmetric acid-induced cell extension leading to gravicurvature.

**Acknowledgment—**We would like to thank Professor William K. Purves in whose laboratory the chromatographic analyses were performed.

**LITERATURE CITED**

10. Iwami S, Y Masuda 1974 Geotropic response of cucumber hypocotyls. Plant...
AUXIN AND SHOOT GRAVITROPISM


16. MULKEY TJ, KM KUZMANOFF, ML EVANS 1981 Correlations between proton efflux patterns during geotropism and phototropism in maize and sunflower.


