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THE OCCURRENCE AND SIGNIFICANCE OF AUXIN IN THE SUBSTRATA OF BRYOPHYTES

By A. R. SHELDRAKE

Department of Biochemistry, University of Cambridge

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Summary

Auxin was detected in samples of substrata supporting bryophytes in a variety of locations in both Britain and Malaya. Activity occurred on chromatograms at zones corresponding to the Rf of indole acetic acid. The range of concentrations found, 0.4–10.4 μg/l, probably represents a two- to five-fold underestimate due to losses during extraction and purification. The amounts of auxin in samples of soil on which bryophytes were not growing were within the same range. The importance of this environmental auxin for the induction of rhizoids in liverworts and for roots of higher plants is discussed.

Introduction

There have been a number of reports concerning the effects of exogenous auxin on bryophytes. On most of the responses studied, auxin has been found to be inhibitory; for example it inhibits thallus growth in *Marchantia* (Kaul, Mitra and Tripathi, 1962), lateral branching in *Splachnum* (MacQuarrie and von Maltzahn, 1959), bud formation in moss protonemata (Mitra and Allsop, 1959) and the development of moss and liverwort gemmae (Narayanaswami, 1956; La Rue and Narayanaswami, 1957). The only well-established positive response is the stimulation of rhizoid formation in liverworts (La Rue, 1942; Moewus and Schader, 1952; Rousseau, 1953; Klingmüller, 1959; Kaul *et al.*, 1962; Maravolo and Voth, 1966). Unlike most of the inhibitory effects, where the levels of auxin used may have been approaching the threshold of toxicity, the induction of rhizoids can be brought about by very low concentrations and may be of physiological importance. This does not necessarily imply that rhizoid production is under the control of endogenous auxin, since the experimentally observed stimulation of rhizoid formation by exogenous auxin could be a normal phenomenon if auxin is commonly present in the natural environments of bryophytes. Soils, particularly those rich in decaying organic matter, are known to contain auxin (Whitehead, 1963). In this paper I present evidence which shows that auxin also occurs in the immediate environment of bryophytes collected from a variety of locations in both Britain and Malaya.

Materials and methods

Bryophytes, collected together with their immediate substrata, were taken to the laboratory in polythene bags. The Malayan specimens were identified by Professor Ann Johnson and the British ones by Mr John Dransfield. The green, living plants were carefully detached from their substratum, 20–60 g of which was then extracted twice for

Table 1. *Auxin content of bryophyte substrata*

Sample	Date	Collected	Location	Species	Auxin (as IAA) µg/kg FW	Auxin (as IAA) µg/l soil soln
A	19 Oct. 1968	Cameron Highlands, Pahang, Malaya	Sloping bank	*Solenostoma* sp.* *Pogonaum microphyllum* (Dz. et Mb.) v.d. B. et Lac.†	2.0	2.9
B			At base of forest tree	*Cephaloziella* sp.* *Bazzania* sp.* *Pallavicinia leveiri* Schffn.*	1.3	1.8
C			Rotting tree trunk	*Acrosporium diminutum* (Brid.) Flsch.†	1.9	2.0
D			Beside path, beneath undergrowth	*Thamnium latifolium* (v.d. B. et. Lac.) Par.†	3.9	4.4
E			Forest floor	*Dumortiera hirsuta* (Sw.) Nees.* *Cephaloziella* sp.*	1.0	1.8
F			Forest floor	*Acroporium diminitum* (Brid.) Flsch.† *Banzzania sp.**	1.5	2.2
G	19 Oct. 1968	Nr Tanah Ratah, Pahang, Malaya	Vertical bank	*Pogonatum junghuhnianum* (Dz. et Mb.) Lac.†	5.4	10.4
H	7 Sept. 1968	Ulu Gombak, Selangor, Malaya	Rock beside stream	Unidentified mosses and liverworts	1.8	3.5
I			Rotting log	Unidentified mosses and liverworts	0.4	0.6
J	13 Sept. 1969	Madingley Wood, Cambridge, England	Rotting log	*Cephalozia bicuspidata* (L.) Dum.* *Lophocolea heterophylla* (Schrad.) Dum.* *Bracythecium rutabulum* (Hedw.) B., S., et G.† *Amblystegium serpens* (Hedw.) B., S., et G.†	0.3	0.4

				Mnium longirostrum Brid.†		
				Bryum capillare Hedw.†		
				Hypnum cupressiforme Hedw.†		
				Eurynchium confertum (Dicks.) Milde†		
K	7 Oct. 1969	Betws-y-Coed, Wales	Wall top	*Plagiochila asplenioides* (L.) Dum.*	1.4	2.1
				Barbula recurvirostra (Hedw.) Dix.†		
				Isothecium myurum Brid.†		
L			On oak tree	*Frullania dilatata* (L.) Dum.*	5.7	8.0
				Isothecium myosuroides Brid.†		
M	7 Oct. 1969	Llanberis Pass, Wales	Boggy moorland ground	*Cephalozia bicuspidata* (L.) Dum.*	0.6	0.7
				Lophozia vetricosa (Dicks.) Dum.*		
				Leucobryum glaucum (Hedw.) Schimp.†		
N			Moorland ground	*Rhacomitrium lanuginosum* (Hedw.) Brid.	0.5	0.6
O	3 May 1970	Hayley Wood, Cambridge, England	Woodland floor	*Mnium hornum* Hedw.†	0.7	1.1
P	4 Oct. 1970	Madingley Wood, Cambridge, England	Woodland floor	*Pellia endiviifolia* Dicks.*	0.9	1.3
				Acrocladium cuspidatum (Hedw.) Lindb.†		
				Eurhynchium swartzii (Turn.) Cum.†		
				Mnium undulatum Hedw.†		
				Thamnium alopecurum (Hedw.) B. et S.†		

* Liverworts.
† Mosses.

2 hours with 200 ml peroxide-free ether at 0° C. After concentration under reduced pressure, the ether extract was partitioned with three aliquots of 0.1 M sodium bicarbonate. After acidification to pH 3 in the presence of methyl orange as internal indicator, the solution was extracted three times by shaking with peroxide-free ether. This ether extract, containing acidic ether-soluble substances, was concentrated and applied to the origin of cellulose thin-layer plates which were developed usually with isopropanol–ammonia–water (10:1:1 v/v/v) but also with other solvents as indicated below. The cellulose was scraped off appropriate zones and placed in small tubes for bioassay. Marker spots of indole acetic acid (IAA) were also run on the chromatograms and were detected by a perchloric acid/$FeCl_3$ spray (Larsen, 1955). In one case, an aqueous extraction method was used. The sample (30 g) was shaken with 300 ml 0.1 M sodium bicarbonate solution at 4° C for 3 hours and centrifuged. The supernatant was partitioned three times with ether to remove basic and neutral ether-soluble substances which were discarded. The aqueous extract was acidified to pH 3 and partitioned three times with ether. Concentration and chromatography of this fraction were conducted as above.

In Malaya, 3-day-old seedlings of *Avena sativa* L. var. Victory (Svalof, Sweden) grown in sand at 25° C were used for the coleoptile straight-growth bioassay of Nitsch and Nitsch (1956). One millilitre of solution was used in each assay tube. In Cambridge, $3\frac{1}{2}$-day-old seedlings of *A. sativa* var. W.W. 16253 (Landskrona, Sweden) grown in vermiculite at 20° C were used for their mesocotyl extension bioassay. Standard curves were prepared using 1–500 ng/ml IAA. The amount of auxin in the samples, determined in terms of IAA equivalents, is expressed on a fresh weight (FW) and concentration basis. The latter was calculated by taking the water content of a sample as the difference between its fresh weight and dry weight after oven-drying for 3 days at 60° C.

Percentage recoveries of auxin were estimated by adding 0.1 μg of [1-^{14}C]IAA (25.6 mCi/mM, Amersham) to samples before extraction. Extraction, partitioning and chromatography were carried out in the usual way. Chromatographic zones corresponding to the Rf of IAA were scraped off and placed in scintillation vials with 3.5 ml of a liquid scintillator (Bray, 1960) in which IAA is readily soluble. An equal quantity of cellulose powder was added to IAA controls and to blanks. The vials were shaken at intervals for 1 hour and the contents allowed to settle before counting using a Nuclear Chicago scintillation counter.

RESULTS

Auxin was detected in all samples. Table 1 shows the amount/kg FW and also the calculated concentration of auxin in solution. A similar range of concentrations (0.4–10.4 μg/l) was found in Malaya and Britain.

An aqueous extract of one sample (O) had an auxin content of 0.3 μg/kg FW, less than half that found by ether extraction.

The auxin content of some soil samples on which bryophytes were not growing was also determined. A sample taken from the top 2 cm of the forest floor near where the Malayan samples E and F were collected contained 1.8 μg/kg FW or 3.5 μg/l in the soil solution. Soil collected in Madingley Wood, Cambridge, in July 1970 contained 4.5 μg/kg FW or 11.0 μg/l in a surface (2 cm) sample and 1.5 μg/kg FW or 6.0 μg/l in a deeper (5 cm) sample less rich in humus.

Auxin activity was detected on chromatograms at an Rf similar to that of IAA, as shown in Fig. 1. Inhibitory substances with a higher Rf were also present in most extracts.

Zones corresponding to the Rf of IAA from chromatograms of extracts of a substratum sample (P) and of a surface soil sample were scraped off and eluted with ether. The eluates were applied to the origins of cellulose thin-layer plates which were developed with four different solvent systems: *n*-butanol–ethanol–water (4:1:1 v/v/v), pyridine–ammonia (4:1 v/v), chloroform–acetic acid (95:5 v/v), and ethanol–water (7:3 v/v). The Rf's of IAA in these systems were 0.90, 0.57, 0.98 and 0.74 respectively. Bioassays

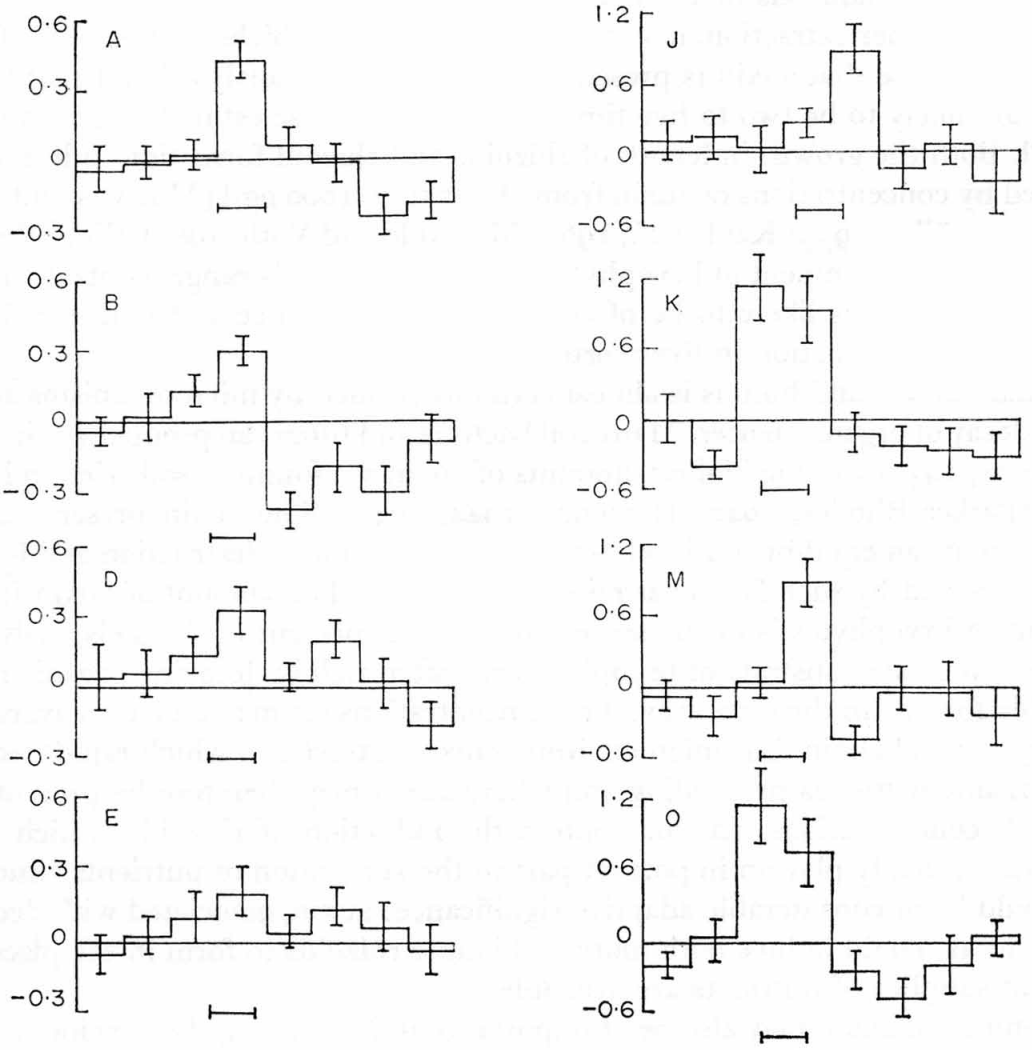

Fig. 1. Auxin activity in zones eluted from chromatograms developed with isopropanol–ammonia–water (10:1:1v/v). The Malayan samples (left) were tested by the coleoptile section bioassay, the British samples (right) by the mesocotyl section bioassay. The extensions (mm) of 4 mm sections are shown together with mean deviations. The positions of marker IAA spots (which varied slightly from time to time) are indicated under each diagram; the origin is shown at the left, the solvent front at the right. See Table 1 for origin of samples.

of zones of these chromatograms showed that in all cases auxin activity occurred at an Rf corresponding to that of IAA. This evidence indicates that the auxin activity was due to the presence of IAA in the extracts.

Estimations of the recovery of auxin, made by adding [1-^{14}C]IAA to samples of soil or substrata before extraction, were 22% for ether extraction and 19% for aqueous extraction.

Discussion

All estimations of auxin based on extraction methods raise problems of interpretation. Recoveries of auxin are usually in the order of 12–25% (Hamilton, Bandurski and

Grigsby, 1961) and these may be overestimates of the recovery of endogenous auxin since they depend on adding auxin to the samples externally. The recoveries of about 20% found in this work suggest that the amounts of auxin in the substrata and soils are likely to be at least five times greater than those estimated. Ether extraction may not be the best procedure for determining the free auxin in the soil since ether could also extract auxin which is not available to the soil solution, for example, any present within the cells of the microflora or fauna; the aqueous method, which yields estimates of auxin content about half those found by ether extraction, may give a truer picture. Whichever method is favoured, the results indicate that auxin is present in all the samples analysed and that the actual amounts are likely to be two to five times greater than those estimated, i.e. in the range 1–50 μg/l. Both the growth in length of rhizoids and rhizoid formation in liverworts are stimulated by concentrations of auxin from about 10 to 1000 μg/l (Moewus and Schader, 1953; Klingmüller, 1959; Kaul *et al.*, 1962; Maravolo and Voth, 1966). Since the amount of auxin in the environment of bryophytes is often within this range, contact with soil or decaying vegetation is likely to be of considerable importance and is in fact known to stimulate rhizoid formation in liverworts (Garjeanne, 1932).

The auxin of soil and humus is almost certainly formed by microorganisms associated with the decay of organic matter. Many soil bacteria and fungi can produce auxin (Roberts and Roberts, 1939) and the highest amounts of auxin are found in soils rich in humus or manure (Parker-Rhodes, 1940; Hamence, 1944, 1946). The auxin present at a given time represents an equilibrium level between formation and destruction and is presumably also affected by such factors as rain and leaching. The amount of auxin in the environment of bryophytes is of the same order as that present in the soils analysed and, since the immediate substrata of bryophytes are often rich in decaying organic matter, it is probably formed in the same way. The concentrations estimated give an average value for environmental auxin, but microenvironments must exist in which rapid decomposition of organic matter is proceeding and where auxin may therefore be present in relatively high concentrations. In this context the induction of rhizoids, which in many bryophytes probably play an important part in the absorption of nutrients (Buch, 1945, 1947), could be of considerable adaptive significance: auxin, associated with decomposition and decay, could induce both more and longer rhizoids to form in the places where the richest supplies of nutrients are available.

Environmental auxin may also be of importance in influencing the development of the roots of higher plants, which are affected by extremely low concentrations of exogenous auxin. The elongation of roots is inhibited by concentrations above about 0.001 μg/l (Whitehead, 1963) while the growth of root-hairs is stimulated by concentrations below 0.1 μg/l (Ekdahl, 1957). The treatment of roots with auxin at these low concentrations thus results in a more dense covering of longer root-hairs (Ekdahl, 1957). Auxin is present in soils in quantities great enough to bring about these effects which, like the induction of rhizoids in liverworts, could well be adaptive significance: auxin, acting as a messenger of decomposition and decay, causes the surface area available for absorption to be increased in those environments where nutrients are abundant.

Although it is well established that auxin is a hormone of vascular plants, there is no convincing evidence that it has a similar rôle in lower plants. Attempts have been made to explain phenomena such as apical dominance in fern gametophytes, mosses and liverworts in terms of auxin formation by meristematic cells, but these have rested much more on analogies with higher plants than on critical evidence. It is indeed far from certain that non-vascular plants such as bryophytes and fern gametophytes normally produce

auxin: no auxin could be extracted from seta tissue of *Pellia* by Overbeck (1934); the thorough investigation of Goedecke (1935) failed to reveal any diffusible auxin in *Marchantia*; qualitative determinations of auxin from fern prothalli (Albaum, 1938) and from liverworts (von Witsch, 1940; Kaul *et al.*, 1962) were performed on non-sterile tissues, where epiphytic bacteria are known to produce very significant amounts of auxin (Libbert *et al.*, 1966); and the detection of auxin in sterile liverwort tissue by Schneider, Troxler and Voth (1967) depended on extraction techniques open to the criticism that the small amounts of auxin found could have been produced during the extraction and purification procedures. Hotta (1959) who worked with sterile fern gametophytes and used orthodox extraction methods, was unable to detect any diffusible or free auxin except in old plants. It seems possible that auxin is not a hormone in non-vascular green plants and that effects such as the stimulation of rhizoid production can be explained in terms of environmental auxin.

The possible adaptive significance of responses to environmental auxin by bryophytes and by roots of higher plants, and the hormonal rôle of auxin in higher plants may be evolutionarily connected. IAA is formed by a wide variety of organisms as a breakdown product of the amino acid, tryptophan; it is produced not only by the microbial decay of organic matter but also by the autolytic processes associated with cell death (Sheldrake and Northcote, 1968a). If lower plants such as liverworts evolved an adaptive response to environmental auxin, then similar responses would be expected when cells of the plant itself died, for example as a result of wounding, since auxin would be released by the dying cells. Rhizoids are in fact induced in liverworts by wounding (La Rue, 1942). With the evolution of the vascular plants, cell death became an integral part of growth and development, because the differentiation of xylem cells involves the death and disappearance of their contents. Auxin, which might previously have been of importance as a chemical messenger from the environment and which was now formed within the plant during the autolysis of differentiating vascular tissue, was in a suitable position to become an endogenous hormone. There is evidence that differentiating xylem tissue is in fact a major source of auxin (Sheldrake and Northcote, 1968b; Sheldrake, 1971); and other sites of cell death in higher plants are also sites of auxin formation (Sheldrake and Northcote, 1968a, b, c). Vascular differentiation in young roots could lead to auxin production as great as that in shoots, but roots contain highly active auxin destroying enzymes (Åberg, 1956) which may account for their low levels of endogenous auxin. If roots contained relatively high amounts of auxin, their great sensitivity to environmental auxin would not be possible.

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