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Changes in auxin activity in tumourous and normal tobacco calluses treated with morphactin IT 3233

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Abstract

The addition of morphactin IT 3233 in 1-40 mg/dm³ concentrations to the medium inhibited the growth *in vitro* of normal and tumourous tobacco calluses. The auxin activity (estimated by the *Avena* coleoptile straight growth test) of the acid ether extracts from these tissues increased. The activity of zone I (R_f 0.2—0.4, 0.5, solvent system: butanol:water:ammonia 10:10:1) in normal tissues increased more intensively than that of zone II (R_f 0.6—0.8, 0.9). In tumourous tissues, however, these changes were smaller and they concerned merely zone I of auxin activity (R_f 0.0—0.5).

It seems that the mechanism of morphactin activity in both kinds of tissue is different. It may be supposed that the excessive accumulation of auxins induces growth inhibition of tissues. A previously found increase in the activity of IAA-oxidase influenced by morphactin might be considered as an adaptation to a higher level of IAA.

INTRODUCTION

Many responses of plants to morphactins, such as disturbances in geotropic reaction and apical dominance and also induction of parthenocarpy, could be connected with the influence of these compounds on the auxin system. The mechanism of their action is ascribed to the inhibition of polar transport of auxins (Krelle, Libbert 1968, Tognoni, Alpi 1969, Naqui 1972, Bridges, Wilkins 1973, Beyer, Quebedeaux 1974) or to the influence on the activity of enzymes connected with IAA metabolism (Khan 1967).

In our previous investigation (Chirek, 1974) dealing with the influence of morphactin IT 3233 on tobacco tissues cultured *in vitro* we found an inhibition of growth of normal callus tissue accompanied by

changes in the metabolism such as a decrease of respiratory activity and protein synthesis with simultaneous protein accumulation and increase in dry matter content and also a stimulation of IAA-oxidase activity. Tumourous tissues were less sensitive to morphactin as related to both growth response and individual metabolic processes. These facts suggest that morphactin could influence the growth of tissues under examination by changing the IAA level in them. In the case of normal callus tissues requiring in vitro exogenous IAA, it was suggested that morphactin makes penetration of this compound from a medium into tissue difficult. Tumour tissues, however — as capable of IAA synthesis — may be less sensitive to morphactin.

The aim of this work was to prove this supposition by way of auxin level determination in normal and tumourous tissues cultured in a medium containing morphactin.

MATERIAL AND METHODS

5-week colonies of tissues: callus from a stem and tumour of bacterial origin from *Nicotiana tabacum* L. cv. White Burley cultured in Murashige and Skoog medium (1962) modified by Linsmaier and Skoog (1965) with morphactin IT 3233 (n-butyl ester of 9-hydroxyfluorene-(9)-carboxylic acid, E. Merck AG) added in quantities of 1, 10, 40 mg/dm^3 were used. IAA (2 mg/dm^3) and kinetin (0.25 mg/dm^3) were also added to the medium prepared for normal callus tissues. The remaining culture conditions were the same as previously (Chirek, 1974).

Samples (10 g) of fresh tissue were extracted for 5-6 hours as previously described (Chirek, 1979). After chromatographic separation auxin activity of the acid ether extracts was estimated by the *Avena* coleoptile straight growth test. This activity is shown in histograms and expressed as per cent of the control increase. The auxin level in µg equivalents of IAA was read from a standard curve and calculated per 1 kg of fresh tissue. Mean values ±s.e. from 5-6 analyses are presented in the table.

RESULTS

Table 1 shows the results (mean values from 5-6 series) representing the growth of normal and tumourous tissues in the standard medium and with morphactin IT 3233 after 5 weeks of culture. During this period both tissues showed a similar growth rate. Under the influence of morphactin growth inhibition took place. In the case of normal tissue this inhibition approached 50%, in the tumourous one — 60%. These

Table 1

Growth of tobacco callus and tumour tissues on medium with morphactin IT 3233

conc. of morphactin mg/dm³		0 (control)	1	10	40
callus	fresh weight per colony (mg)	1203 ± 58	1125 ± 79	1010 ± 40*	560 ± 53*
	% of control	100	94	84	47
tumour	fresh weight per colony (mg)	1248 ± 148	1007 ± 121	825 ± 91*	462 ± 58*
	% of control	100	82	67	38

* — difference significant at 5% level.

differences were statistically significant at 10 and 40 mg/dm³ morphactin concentrations. The tumourous tissue proved to be more sensitive than that examined previously (Chirek — 1974, part I) where the same morphactin concentration induced only 26% of inhibition. This argues a certain mutability of the tissue strain maintained *in vitro* since 1962 and the difference in age of the compared tissues was nearly 6 years.

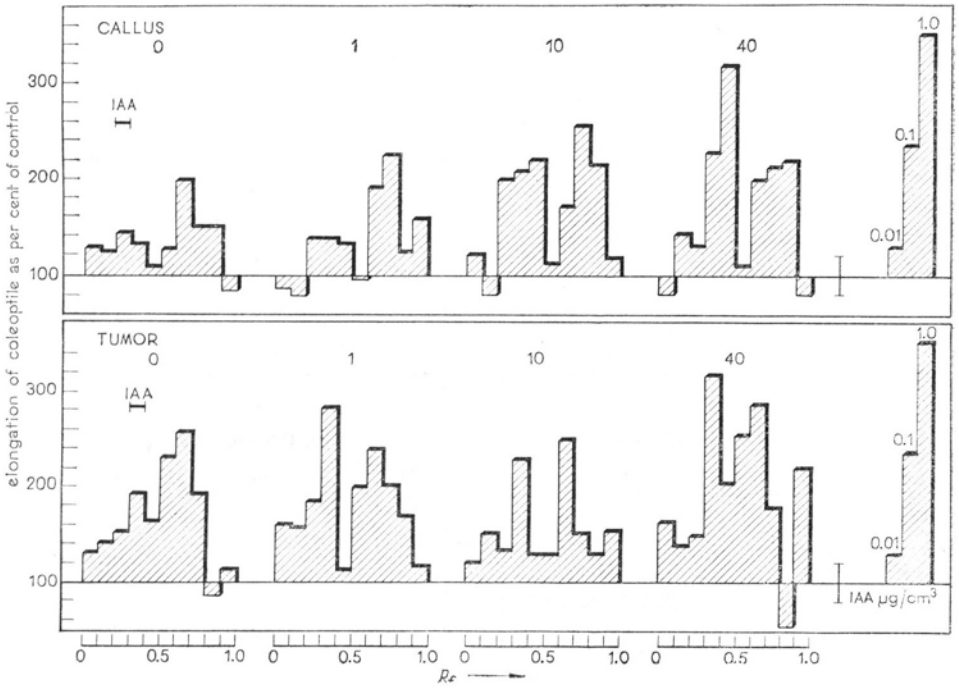

Fig. 1. Auxin activity of acidic ether extracts in tobacco tissues cultured with IT 3233 (numbers: 0, 1, 10, 40 indicate concentrations of morphactin in mg/dm³). Elongation produced by standard solutions of IAA is indicated on the right of the histogram. Vertical lines crossing abscissa represent confidence limits (95%) for control in the Student's t-test.

Auxin activity of normal and tumourous tissues depending on the concentration of morphactin in the medium is presented in the histogram (Fig. 1) showing results of the test of one tissue series. On the basis of several series of determinations the presence of two main active zones was found:
for the normal callus — zone I at 0.2 — 0.4, 0.5 R_f, zone II at 0.6 or 0.5 — 0.8, 0.9 R_f;
for the tumourous callus — zone I: 0, 0.1 — 0.5 R_f, zone II: 0.5 — 0.8, 0.9 R_f.

Under the examined conditions of separation the standard IAA in particular experiments takes a position showing R_f 0.27 — 0.36, which corresponds to a maximum activity of zone I.

Table 2

Auxin activity of extracts from tobacco tissues cultured for 5 weeks on medium with morphactin IT 3233 (in µg-equ. IAA/kg fresh weight)

tissue	callus		tumour	
conc. of morph. mg/dm³	zone of activity R_f	µg-equ. IAA	zone of activity	µg-equ. IAA
0	I 0.2—0.4, 0.5	9 ± 1.5	I 0, 0.1—0.5	30 ± 8
	II 0.5, 0.6—0.8, 0.9	18 ± 4.5	II 0.5—0.8, 0.9	58 ± 10
	total (I+II)	27 ± 6	total (I+II)	88 ± 17
1	I 0.2, 0.3—0.5	12 ± 4	I 0—0.4, 0.5	68 ± 13
	II 0.5, 0.6—0.9, 1.0	29 ± 6	II 0.5, 0.6—0.9, 1.0	64 ± 10
	total	41 ± 7.5	total	132 ± 21
10	I 0.2, 0.3—0.5	31 ± 10	I 0, 0.1—0.5	85 ± 31
	II 0.5, 0.6—0.9, 1.0	43 ± 11	II 0.5, 0.6—0.9, 1.0	68 ± 12
	total	74 ± 21	total	153 ± 35
40	I 0.1—0.5	125 ± 32*	I 0—0.5	115 ± 26*
	II 0.5, 0.6—0.8, 0.9	63 ± 7*	II 0.5—0.8, 0.9	90 ± 19.5
	total	188 ± 35*	total	205 ± 44*

* — difference significant at 5% level.

Tumourous tissue distinct from the callus shows a clear auxin activity at R_f 0.5 — 0.6 which corresponds to the position of the standard indole-3-butyric acid (IBA). Bayer (1969) found a high IBA content

in the stems of tobacco tumour-forming hybrids. In the presence of morphactin an increase in the auxin activity of tissues, particularly normal, and also an enlargement of zone II to the front of the chromatogram took place.

Mean results of the tests calculated in relation to the µg-equivalent of IAA are given in table 2. Similarly as previously (C h i r e k, 1979) the auxin level was nearly 3 times higher as compared to that in normal tissue and the auxins of zone I make about $\frac{1}{3}$ of the total in both tissues types.

In the normal callus tissues cultured in a medium with 10 and 40 mg morphactin/dm³ the level of auxins in zone I increased 3 and 14 times, respectively, and that of zone II — 2.5 and 3.5 times.

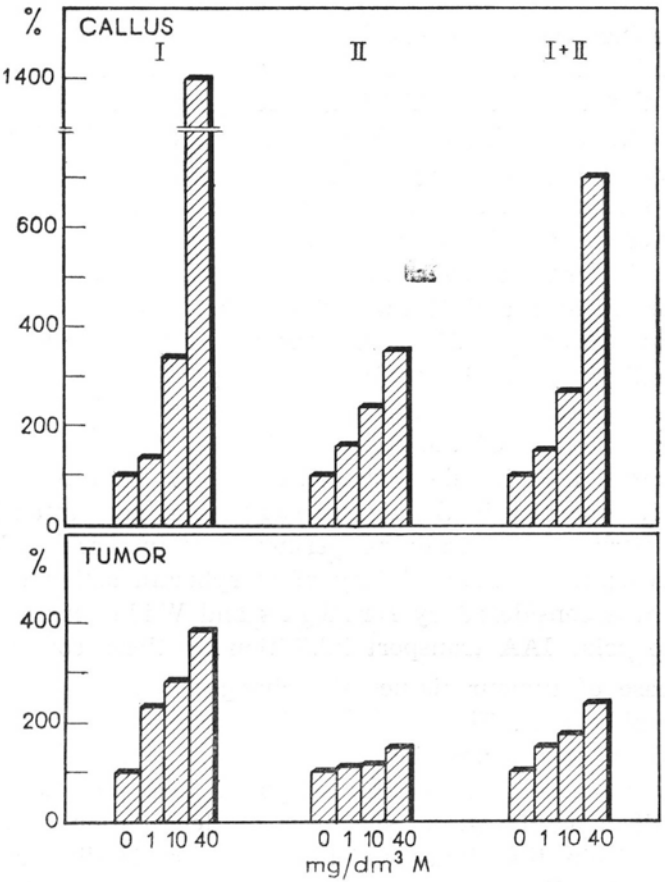

Fig. 2. Changes of auxin activity in tobacco tissues treated with IT 3233 (M) in per cent of control. I, II — zones of activity, as in table 2. 0, 1, 10, 40 — concentrations of morphactin in mg/dm³.

These changes were lesser in tumour tissues; the activity of zone I increased 2-4 times at 1-40 mg/dm^3 concentrations and that of zone II — slightly, only at the highest morphactin concentration — higher by about 50%. These changes expressed as per cent of control are shown in the diagram (Fig. 2).

The total auxin quantity in tumourous tissue increased 2.5 times at the highest morphactin concentration, at a normal one, however, as much as 7 times. In tumour tissue mainly the auxin level of zone I increased, in normal tissue — also that of zone II.

DISCUSSION

In our previous paper (Chirek, 1974) concerning the action of morphactin on tobacco tissues cultured *in vitro,* a positive correlation between the increase in IAA-oxidase activity in tissues and the degree of growth inhibition was found. This could suggest a decrease of IAA content as the cause of inhibition. The present investigations have shown, however, a considerable increase in the auxin level, particularly in zone I containing probably IAA, in normal and tumourous tissues growing in media with higher concentrations of morphactin. Thus, in normal tissues the activity of zone I considerably increased and that of zone II a little less. However, the proportions between zones I and II were maintained at 1 and 10 mg/dm^3 of morphactin but at 40 mg/dm^3 they were significantly shifted in favour of zone I. This could point to essential disturbances in auxin metabolism. The sevenfold increase in the auxin level as well as changes in these proportions could damage the regulatory ability of the tissue and lead to a significant growth limitation. Since IAA is taken by callus tissues from the medium, an increase in its quantity in the tissues suggests a more intensive uptake as a result perhaps of membrane permeability increase under the influence of morphactin. A possibility of morphactin action on plasmatic membranes was considered by Bridges and Wilkins (1973) as the cause of the polar IAA transport inhibition by these compounds.

In the case of tumour tissues the changes concerned the auxin of zone I, probably IAA. The auxin level of zone II was changed only slightly and in this connection the proportions between them were considerably disturbed. It can be supposed that morphactin changes IAA metabolism acting on its synthesis or degradation or disturbs its distribution within the tissue which, as known, is differentiated and contains what is called centres of actively proliferating cells and larger cells showing a slighter proliferation activity. According to Kupila (1963) the differentiation of tumour cells is a reflection of various activations of the systems producing growth regulators.

The auxin accumulation (presumably IAA) found in this work in tobacco tumour tissue could be the cause of growth inhibition. It is known that tumour tissues synthesise auxins in optimum quantities for growth, and an increase in their level leads to growth limitation (de Ropp, 1947).

In the light of these data the increase in the activity of IAA-oxidase in tobacco tissues treated with morphactin (particularly distinct in normal callus tissues) found previously (Chirek, 1974, part IV) could be an adaptation of the tissue to the raised IAA level in them. A high activity of IAA oxidation could lead consecutively to accumulation of the product of this reaction — 3-methyleneoxyindole which — as it follows from Gaspar's (1973) experiments is a growth-inhibiting factor.

Beyer and Quebedeaux (1974) found that the morphactin-induced partenocarpy in the cucumber ovary due to auxins accumulation is a result of inhibition of their transport.

In the case of the tissues under examination it seems that the mechanism of morphactin action in callus tissue is different from that in tumours. Various changes in the auxin activity of tissues and the degree of growth inhibition point to this. A different IAA source in the case of normal and tumourous callus, as well as its higher level found in tumourous tissue can condition this mechanism.

There are no data concerning the action of morphactins on tumour tissues. On the other hand, as Ogura (1975) reported, in the tobacco callus morphactin could replace auxin in the induction of morphogenesis. Either this effect was induced by the stimulation of the auxin synthesis or morphactin itself influences similarly as auxin remains to be explained.

It is imposible at present to state the mechanism of morphactin activity. The Schneider's opinion (1970) that this action is multidirectional seems to be continually right.

In order to elucidate the mechanism of the action of morphactins on normal and tumourous tissues it would be necessary to apply modern methods of auxins identification as gas and liquid chromatography and determination of the activity of enzymes connected with IAA synthesis and degradation.

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Zmiany aktywności auksynowej w kalusach tumorowych i normalnych tytoniu traktowanych morfaktyną IT 3233

Streszczenie

Wprowadzenie morfaktyny IT 3233 w stężeniach 1-40 mg/dm^3 hamowało wzrost tkanek kalusowych normalnych i tumorowych tytoniu w hodowli *in vitro*. Aktywność auksynowa (oznaczana testem elongacyjnym koleoptyli owsa) kwaśnych

ekstraktów eterowych z tych tkanek wzrastała. W tkankach prawidłowych znacznie zwiększała się aktywność I strefy (R_f 0.2-0.4, 0.5, rozwijacz: butanol: woda: amoniak, 10:10:1), mniej — II strefy (R_f 0.6-0.8, 0.9). W tkankach tumorowych zmiany były mniejsze i dotyczyły tylko I strefy aktywności auksynowej (R_f 0-0.5).

Wydaje się, że mechanizm działania morfaktyny na te tkanki jest różny. Przypuszcza się, że nadmierna akumulacja auksyn jest przyczyną zahamowania wzrostu tkanek, a stwierdzone we wcześniejszych badaniach podwyższenie aktywności oksydazy kwasu indolo-3-octowego mogło być adaptacją do wyższego poziomu kwasu indolo-3-octowego (IAA).