

Physiological and biochemical effects of morphactin IT 3233 on callus and tumour tissues of *Nicotiana tabacum* L. cultured in vitro.

IV. IAA oxidase activity. Oat coleoptile biotest

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Abstract

IAA oxidase activity in callus and tumour tissue of tobacco subjected to the action of morphactin IT 3233 for shorter and longer periods was determined. Control tumour tissue shows an activity higher by about 40 per cent as compared with that of callus tissue. Morphactin applied for a short time (24-h incubation) does not change the activity of the enzyme. When application is prolonged, a considerable enhancement (up to 140%) of the enzyme activity in callus tissue is observed in dependence on the morphactin concentration. In tumour tissues the activity is stimulated by 45 per cent as compared to control. Oat coleoptile elongation growth induced by IAA is limited to 40 per cent when morphactin is added in the concentrations used for tobacco tissue cultures. The possibility of the morphactin action on tissue growth via IAA metabolism is discussed.

INTRODUCTION

A number of effects induced by morphactins may be explained by various kinds of interaction of the latter with auxins.

For instance the abolition of apical dominance and induction of development of the lateral buds by morphactin are ascribed by Mann et al. (1966) to the antagonistic action of this compound towards IAA. Tognoni and Alpi (1969) believe, on the basis of their studies, that the mechanism of effects of this type consists in the inhibition of polar transport of IAA from the terminal bud. The experiments of Bopp (1969), Krelle and Libbert (1968b) and Parups (1970) also indicate an inhibition of IAA transport by morphactin. Ziegler (1970) is of the opinion that the observed disturbances in the polarity of cell division may be explained by disorders in the polar transport of auxin.

Schneider (1970) suggests that the influence of morphactin on the frequency of cell divisions (inhibition in the growth apices — Ringe et al. 1967, Schraudolf 1967, Denffer et al. 1969; stimulation on plant explantates — Alleweldt and

Bourquin 1967, Julliard 1966, Schneider 1967) points to their interaction with the cytokinin-auxin system.

In tests with *Avena* and *Triticum* coleoptiles morphactin (chlorflurenol) in low concentrations (10^{-5} — 10^{-7} M) produced auxin-like effects (Krelle, Libbert 1968a, Harada 1969). On the other hand, Bopp (1969) noted a reduction by 50 per cent of IAA-induced elongation growth of *Avena* coleoptile by this substance in a concentration of 10^{-5} g/cm³.

The investigations of Vogt (1968 quoted after Schneider 1970) and of Ziegler et al. (1969) indicate a depression of the IAA level in tissues treated with morphactin. The cause of this may, according to the cited authors, lie in a limitation of IAA synthesis or a change in the activity of the enzymes oxidizing IAA. These changes have been demonstrated by several authors (Khan 1967 — in *Avena* seedlings with disturbed geo- and phototropism, (Vogt 1968 — in pea seedlings, Zalewska and Saniewski 1968 — in ageotropically growing pea seedlings).

Thus, the interaction of morphactins with IAA may occur in various ways — by influencing IAA synthesis or the set of enzymes regulating the level of this hormone, or else by disturbing the normal auxin distribution in the plant.

The aim of the present study was to compare the IAA oxidase activity in callus and tumour tissues of tobacco treated with morphactin. These tissues differ by their sources of auxin — for tumour tissues it is endogenous, and for the callus ones — exogenous. In earlier investigation (Chirek — parts I, II, III, in press) an important influence of morphactin on the growth and metabolism of callus tissue was established, and a much slighter effect on tumour tissue. It seemed, therefore, purposeful to verify whether IAA metabolism undergoes similar changes and may be attributed to other effects of morphactin.

Another aim was to check how morphactin IT 3233 in the concentrations applied to tobacco tissue cultures affects the growth of oat coleoptile in the auxin biotest.

MATERIAL AND METHODS

I. The material for investigation of β -indolylacetic acid oxidase activity consisted of tumour and callus tissue of tobacco cultured in the conditions previously described (Chirek — part I, in press), a part of the control tissues was, however, additionally incubated for 24 h with morphactin solution (1, 10, 40 mg/dm³ concentrations) in 2 per cent glucose.

IAA oxidase activity was determined in extracts from the particular types of tissues and substrates by the manometric method (Stutz 1957, Morgan and Hall 1963, Rennert and Knypl 1967).

Weighed 1-g samples representing several colonies were ground with 10 cm³ of cooled 0.1 M phosphate buffer (NaH_2PO_4 — Na_2HPO_4), pH 6.1. The filtered homogenate four times diluted (1 cm³) was incubated with the buffer (1 cm³ 0.2 M), cofactors (MnCl_2 and 2,4-dichlorophenol — 3 μ moles of each) and substrate (30 μ M IAA in 1 cm³) in Warburg vessels. The measurements of oxygen uptake were

conducted in a Warburg apparatus at 30°C for 3 h at 15-min intervals. The results (in mm^3 of O_2) were converted to 100 mg fresh tissue mass. For each tissue extract the measurements were performed in 4 replications, and the experiments were repeated 3—4 times at about 2-month intervals.

II. The oat coleoptile biotest was carried out after Bentley (1962) in order to establish the influence of morphactin IT 3233, in the concentration applied to tobacco tissue culture, on IAA-induced elongation growth. The material was obtained from oat caryopses of the variety Antoniński późny, germinated in darkness. After germination the seeds were irradiated with red light to stimulate the growth of the coleoptiles. The 10-mm cylinders (deprived of the growth apex) were incubated for 24 h at 25°C) in test solutions containing 2 per cent glucose, phosphate-citrate buffer, pH 5 (ca. 0.07 M), IAA in a $1 \mu\text{g}/\text{cm}^3$ concentration and morphactin in 1, 5, 10, 20 and 40 $\mu\text{g}/\text{cm}^3$ concentrations. The tests were performed with IAA alone, morphactin alone and both these substances jointly and with controls without growth regulators. The length increment of the cylinders is illustrated in diagram 3.

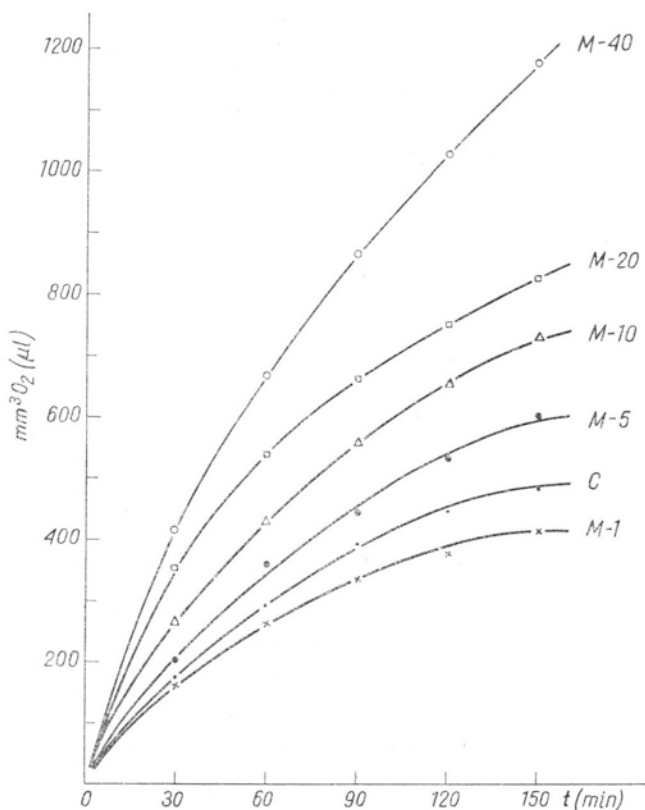


Diagram 1. IAA oxidase activity in callus tissues of *Nicotiana tabacum* L. subjected to the action of morphactin IT 3233 in culture in vitro. C — control (without morphactin). M-1...M-40 — morphactin in concentrations of 1...40 mg/dm^3

RESULTS

I. IAA oxidase activity

Callus. IAA oxidase activity in callus tissue cultured for 5 weeks on a medium with morphactin is shown in diagram 1.

A slight decrease (ca. 10%) in the activity of the enzyme in the tissues is observed at a morphactin concentration of 1 mg/dm³ as compared with the control, and

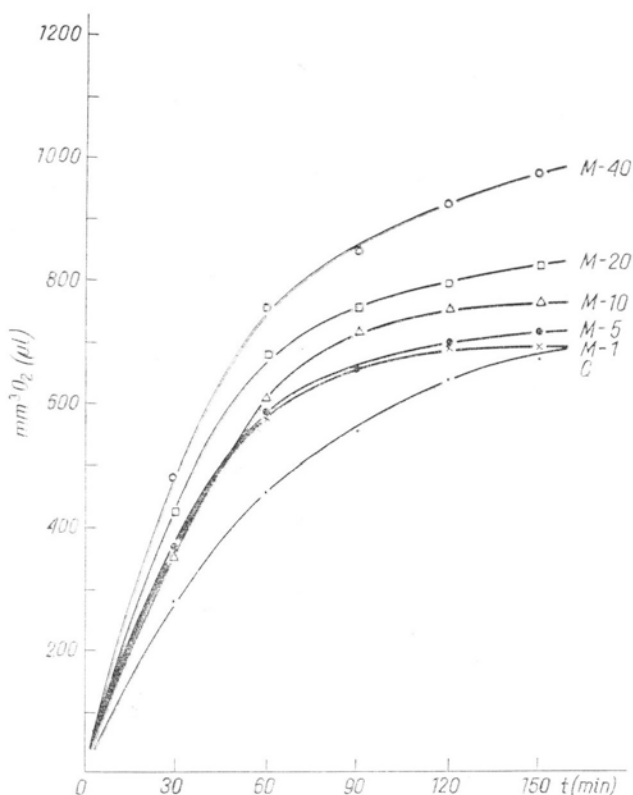


Diagram 2. IAA oxidase activity in tumour tissues of *Nicotiana tabacum* L. subjected to the action of morphactin IT 3233 in culture in vitro. Notations as in Diagram 1

further an increasing enhancement at concentrations of 5–40 mg/dm³. The IAA oxidase activity is about 140, 70 and 50 per cent higher as compared with the control at concentrations of 40, 20 and 10 mg/dm³, respectively (after 150 min of reaction).

When morphactin was applied for a short time (24-h incubation) to callus tissue, no significant changes were noted in IAA oxidase activity in them. The results of determinations are summarized in table 1.

Neither does incubation of control tissues in 2 per cent glucose affect the activity of the enzyme as compared with that in tissues newly separated from the medium.

Table 1

IAA oxidase activity in callus tissues of *Nicotiana tabacum* L. incubated for 24 h with morphactin IT 3233. Results in mm² of O₂/100 mg fresh tissue mass

Time in min.	C	M-1	M-10	M-40
30	200	185	198	150
60	316	310	315	268
90	398	393	397	358
120	462	475	459	434
150	508	516	509	476

C — control (without morphactin)

M-1.....M-40 — morphactin in 1....40 mg/dm³ concentrations

It would seem, therefore, that morphactin does not interact directly with the enzyme and that only prolonged treatment of the tissues with this substance leads to changes in their metabolism, manifested among other symptoms by changes in IAA oxidase activity.

If we refer the IAA oxidase activity to the growth of callus tissue on a medium with morphactin (Chirek — part I, in press), a distinctly negative correlation is seen between these values; a diminution in growth is associated with enhanced IAA oxidase activity.

Tumour. In control tumour tissues the activity of the enzyme was about 40 per cent higher than in analogous callus tissues. Exposure of long duration to morphactin also stimulates the activity of the enzyme but much less than in the callus tissue.

A 40 mg/dm³ concentration enhances enzyme activity by about 45 per cent, 20 mg/dm³ enhances it by 20 per cent. The widest differences in activity in the particular tissues are visible in the first hour of reaction, they diminish later, particularly at lower morphactin concentrations. Under a short lasting action of morphactin — similarly as in callus tissues — no significant differences were found in IAA oxidase activity (table 2).

Table 2

IAA oxidase activity in tumour tissues of *Nicotiana tabacum* L. incubated for 24 h with morphactin IT 3233. Results in mm³ of O₂/100 mg fresh tissue mass

Time in min.	C	M-1	M-10	M-40
30	248	235	217	215
60	401	404	345	358
90	580	551	458	480
120	683	659	560	661
150	803	778	654	753

C—control (without morphactin)

M-1.....M-40 — morphactin in 1...40 mg/dm³ concentrations

II. Biotest with oat coleoptile

The results of the biotest (diagram 3) indicate that morphactin inhibits both endogenous and IAA-induced elongation growth of the oat coleoptile. The degree of inhibition increases with morphactin concentration maximally to 56 per cent of endogenous and 39 per cent of IAA-induced growth.

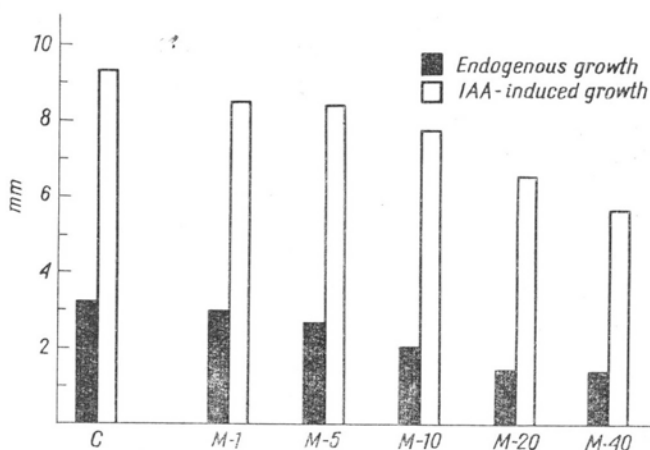


Diagram 3. Oat coleoptile growth in auxin biotest. C — control (without morphactin). M-1...M-40 samples with morphactin IT 3233 in concentrations of 1...40 mg/cm³

These results prove that morphactin itself is a growth inhibitor, and the addition of IAA partly reverses its action. It is possible that morphactin renders difficult IAA uptake and penetration and in this way decreases its influence on growth.

DISCUSSION

According to Galston (1967) the set of enzymes oxidizing IAA regulates physiologically the optimal level of this compound in the tissues.

A relationships between the activity of these enzymes and the extent of growth has not been unequivocally established. Halevy (1963) claims that growth retardants enhance IAA oxidase activity. Also Goven and Tomer (1971) noticed that seselin which inhibits root growth in a number of plants enhances at the same time the activity of peroxidase and IAA oxidase. Knypl and Rennert (1967) found, on the other hand, that the action of CCC and Phosphon-D on cucumber seedlings is not necessarily associated with their action on IAA oxidase. Meudt (1970) speaks even of a positive correlation between growth and IAA oxidase activity in tobacco hybrids. He believes that the IAA oxidase set may function as a part of the mechanism activating auxin.

The present author in her own investigations noted a higher IAA oxidase activation in tumour tissues than in callus tissues of tobacco. The tumour tissues grew somewhat slower than the callus. Morphactin caused growth inhibition in callus tissues (Chirek — part I, in press), and at the same time enhanced IAA oxidase activity. In tumour tissues IAA oxidase activity was considerably less stimulated by morphactin than in callus tissues. A distinct correlation could not be found here between the action of morphactin on growth and the enzyme activity. Growth was inhibited in 20—25 per cent by concentrations of 5—40 mg/dm³ (Chirek — part I, in press), and IAA oxidase was stimulated above 20 per cent only at morphactin concentrations of 20 and 40 mg/dm³.

On the basis of these results it would seem that the influence of morphactin on growth in the tissues investigated is associated with the activation of IAA oxidase in them, differently however, in callus and tumour tissues. It is possible that the auxin uptake from the substrate (in the case of callus tissues) or the penetration of these substances within the tissues are disturbed by morphactin. The depression of the respiratory activity in callus tissues under the influence of morphactin (Chirek — part II, in press) may be the cause of limited auxin uptake from the substrate. Deep changes in the metabolism of callus tissue point to a significant disturbance of the regulating mechanisms. It is probable that the source and level of auxin play here an important role. Changes in the auxin level may produce an adaptation of the enzymes oxidizing IAA.

Lee (1971) established that IAA oxidase activity in tobacco callus cultures was bound with at least two groups of isoenzymes differing in electrophoretic mobility and the reaction to growth substances. IAA in low concentrations enhanced the formation of fast-moving isoenzymes, and in higher concentrations increased the level of slow-moving isoenzymes.

It is not excluded that, in tissues treated for a longer period with morphactin, specific isoenzymes formed in these tissues owing to disturbances of IAA metabolism are responsible for the changes in IAA oxidase activity. The changes in IAA level seem to be more pronounced in callus tissues. The reason of this may lie in the fact that IAA is exogenous for them. The results of the auxin test also indicate disturbances in the activity of exogenous auxin caused by morphactin, perhaps by decreasing the ability of the tissues to take it up from the substrate.

The slight changes in the metabolism and growth of the tumour tissues prove that IAA synthesis within these tissues is a factor which protects them from the action of morphactin.

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*Fizjologiczne i biochemiczne efekty działania morfaktyny IT 3233 na tkanki
— kalusową i tumorową Nicotiana tabacum L. w hodowli in vitro*

IV. Aktywność oksydazy IAA. Biotest koleoptyla owsa

Streszczenie

Oznaczono aktywność oksydazy IAA w tkankach kalusowych i tumorowych tytoniu poddanych krótko- i długotrwałemu działaniu morfaktyny IT 3233. Kontrolna tkanka tumorowa wykazuje wyższą o ok. 40% aktywność enzymu w porównaniu do kalusowej. Morfaktyna przy krótkotrwałym działaniu (24 godz. inkubacji) nie zmienia aktywności enzymu. Przy długotrwałym — obserwuje się silną stymulację aktywności enzymu w tkankach kalusowych — do 140% (zależnie od stężenia morfaktyny).

W tkankach tumorowych stymulacja dochodzi do 45% w porównaniu do kontroli.

Indukowany przez IAA wzrost wydłużeniowy koleoptyli owsa ulega ograniczeniu do 40% przy jednoczesnym zastosowaniu morfaktyny w stężeniach używanych do hodowli tkanek tytoniu.

Rozważa się możliwości oddziaływania morfaktyny na wzrost tkanek poprzez metabolizm IAA.