

Physiological and biochemical effects of morphactin IT 3233 on
callus and tumour tissues of *Nicotiana tabacum* L. cultured *in vitro*
III.* Transamination processes catalysed by aminotransferase
L-alanine: 2-oxoglutarate

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Abstract

An active alanine transaminase was found both in callus and tumour tissues of tobacco. The enzyme is more active in the latter tissue, and the reaction balance is strongly shifted towards alanine production, while in callus tissue towards glutamic acid formation. Morphactin applied to the tissue cultures stimulates markedly the enzyme activity only in callus. A negative correlation was observed between the intensity of transamination processes and enhanced synthesis of proteins in the tissues studied. Morphactin disturbs nitrogen metabolism in the callus tissue. Tumour tissue is more resistant to the action of this substance. The different hormonal activities in these tissues may be the cause of the different effects of morphactin.

INTRODUCTION

In previous studies (Chirek — parts I and II, in press) a significant inhibition of the callus tissue growth in tobacco was noted. It was associated with arise in dry mass and protein content and with depression of the intensity of protein synthesis when morphactin IT 3233 was applied to *in vitro* cultures. In tumour tissue the effect of morphactin was much less pronounced.

The processes of transamination, an important step in the synthesis of amino acids, are closely bound with growth, development and protein synthesis. The main role is played here by glutamic acid as a link binding nitrogen and respiratory, and hydrocarbon and lipid metabolisms (Braunstein 1958) and purine synthesis (Meister 1957).

It therefore, seemed purposeful to verify whether the action of morphactin on tissue growth in tobacco is connected with the regulation of transamination processes with the participation of so important a metabolite as glutamic acid.

* The work made in Department of Pharmaceutical Botany of Medical Academy of Łódź.

MATERIAL NAD METHODS

The material consisted of callus and tumour tissues of tobacco cultured for 5 weeks in the conditions previously described (Chirek — part I in press).

The intensity of the transamination processes catalysed by the extracts from the investigated tissues was expressed in terms of the amount of the amino acid formed in the reaction, which, after a chromatographic separation, was colorimetrically determined.

Analytical procedure as applied in the works of Gubański (1958) and Chirek (1967) was as follows: 2-g weighed samples of tissues (representing several colonies) were ground in a mortar with 5 cm³ of 1/15 M phosphate buffer, pH 7.6 after Sorensen, for tumour tissues, and pH 8 for callus tissues. The homogenate was centrifuged at 500 g for 10 min and the supernatant (in 1-cm³ doses) was incubated with the substrate (0.5 cm³ of each) for 1 h at 30°C. Alanine with α -ketoglutaric acid (I) and glutamic acid with pyruvic acid (II) were used as the reaction substrates in the form of 0.02 M aqueous solutions (the acids were neutralized to pH 7 with 1 M NaOH).

Both these reactions are catalysed by alanine transaminase (KE 2.6.1.2. amino-transferase: L-alanine 2-oxoglutarate), which acts reversibly.

The reaction was interrupted by adding 5 cm³ of absolute ethanol. For each kind of tissue control experiments were carried out in which the enzyme was inactivated at the beginning by adding 5 cm³ of absolute ethanol. After incubation the suspension was centrifuged (see above) and the supernatant was subjected to the chromatographic analysis by ascending chromatography on Whatman no. 3 paper. The liquid was placed linearly in the amount of 0.5 cm³ for reaction I and 0.3 cm³ for reaction II. The chromatograms were developed for 8 h (after previous conditioning) in a phenol-water (4:1) phase. They were dried under air flow for 16 h and shortly in a drier at 65°C.

For developing the colour of the amino acid spots the chromatograms were immersed in 0.5 per cent ninhydrin solution in acetone and left to stand at room temperature for 24 h (Cramer 1962).

For quantitative determinations of the amino acids formed in the transamination reaction, the colorimetric method of Lisitzki and Laurent in the modification of Krietowich and Uspienskaya (1958) was applied. The compounds of a purple-blue colour, formed as the result of reaction of the amino acid with ninhydrin, were eluted from the paper with a 0.5 per cent cadmium chloride solution in 40 per cent methanol (2 cm³ of 0.5% cadmium chloride and 4 cm³ of 40% methanol) in the dark for 2 h. Extinction of the pink complex formed was measured in a chinese photocolorimeter equipped with a no. 50 (green) filter. The measurements were performed in reference to a control sample. The amino acid content was read from standard curves plotted for alanine (up to 30 μ g) and glutamic acid (up to 40 μ g). In the given range they fulfilled Beer's law.

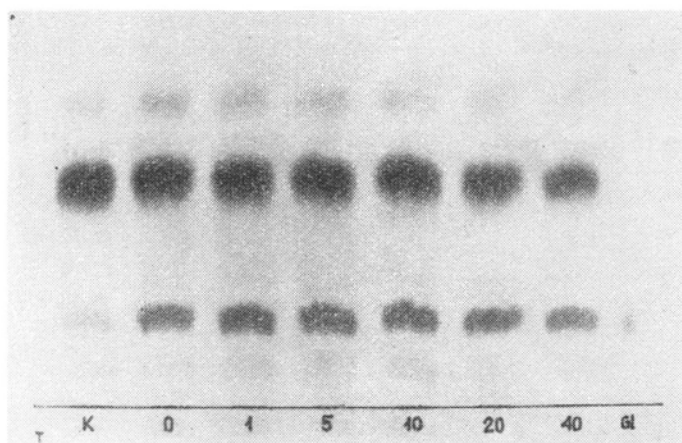


Photo 1. Tumour. Transamination in the system: alanine + α -ketoglutaric acid \rightarrow glutamic acid (Gl). Notations C — control sample — enzyme inactivated: 0, 1, 5, ... 40 — samples with active enzyme, the figures denote morphactin concentrations in the tissue substrate (mg/dm^3)

The results were converted to μmoles of the amino acid formed in the course of 1 h with the participation of 1 cm^3 of a tissue extract. The analyses were replicated 3—4 times.

RESULTS

The photographs of the chromatograms of tumour tissues extracts (Photos 1, 2) and callus tissue extracts (Photos 3, 4) show that in the tissues examined transamination processes occur, leading to the formation of glutamic acid at the cost of the alanine added (Photos 1, 3) and of alanine from the glutamic acid added

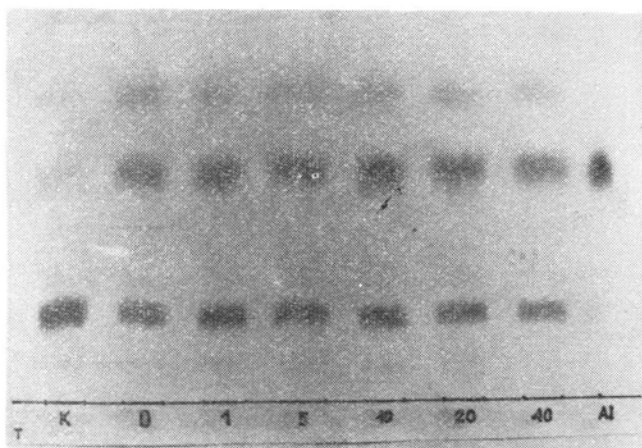


Photo 2. Tumour. Transamination in the system: glutamic acid + pyruvic acid \rightarrow alanine (Al). Notations as in photo 1

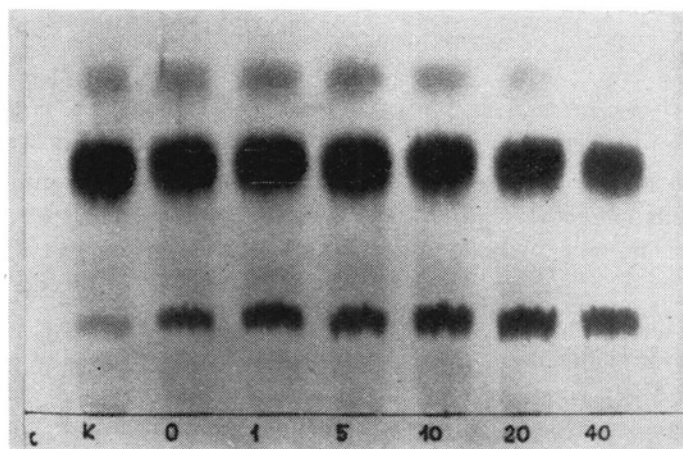
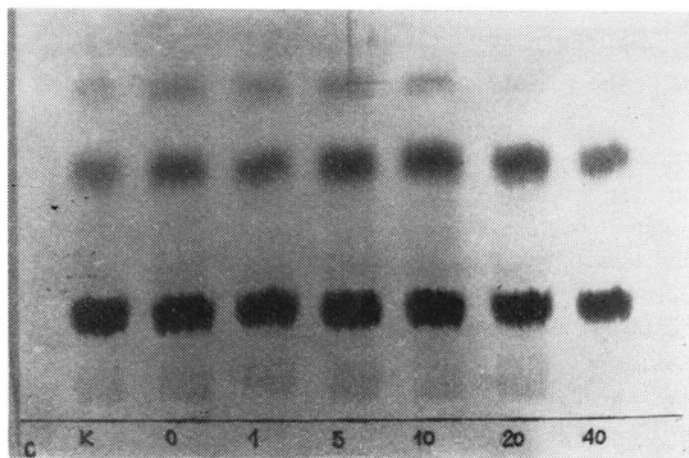


Photo 3. Callus. Transamination in the system: alanine + α -ketoglutaric acid \rightarrow glutamic acid. Notations as in photo 1

(Photos 2, 4). The control, sample (C), where the enzyme was inactivated at the beginning, shows a considerable diminution of the quantity of the above mentioned amino acids as compared with the samples with an active enzyme. Moreover, the colour intensity of the amino acid spots arising from tissue extracts, particularly those from callus (Photos 3, 4) cultured on a medium with and without morphactin in various concentrations seems to indicate a different intensity of these processes in these tissues.

The results of quantitative determinations of the amino acids formed in transamination processes are summarized in the table. These data demonstrate that the control tumour tissue as compared with callus tissue is characterized by a higher intensity of both reactions, and particularly of reaction II leading to alanine for-



Phot 4. Callus. Transamination in the system: glutamic acid + pyruvic acid \rightarrow alanine. Notations as in photo 1

Table 1

Transamination activity in *Nicotiana tabacum* L. tissues subjected to the action of morphactin IT 3233 (M) in culture *in vitro* (Results in μ moles)

Tissues	Medium Results	C	M-1	M-5	M-10	M-20	M-40
Callus	I glutamic acid	1,36	1,11	1,62	2,12	2,44	2,42
	II alanine	0,94	0,71	1,57	1,70	2,01	1,70
Tumour	I glutamic acid	1,84	2,25	2,30	2,24	2,23	2,28
	II alanine	3,53	3,45	3,74	3,74	3,50	3,58

C — control

M-1.....M-40—morphactin in 1.....40 mg/dm² concentrations

mation. The intensity of this reaction is 4 times higher in the tumour tissues. It results from a comparison of reactions I and II in tumour tissue that the latter reaction is almost two times more intensive. In the callus tissue, on the other hand, the main reaction is glutamic acid formation (reaction I), prevailing over alanine formation by about 50 per cent.

In tumour tissues cultured on a substrate with morphactin no significant changes were noted in the transamination processes under investigation. Process I is stimulated only in 25 per cent, and process II does not show any difference as compared with that in control tissues.

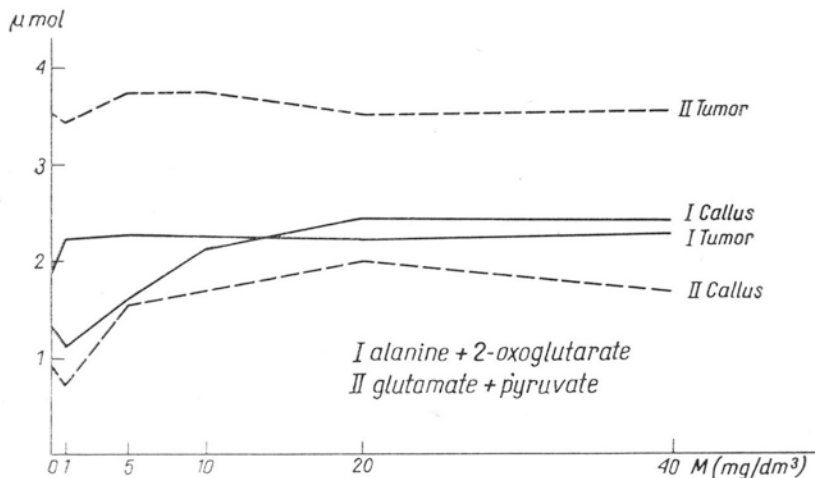


Diagram — Transamination activity in *Nicotiana tabacum* L. tissues subjected to the action of morphactin IT 3233 (M) in culture *in vitro*

In the callus tissues a slight inhibition of both reactions is first observed at 1 mg/dm³ concentration of morphactin, and then a stimulation. At morphactin concentrations of 10, 20 and 40 mg/dm³ the intensity of the processes increases within the limits of 50—110 per cent as compared with the controls. The changes are shown in the diagram.

DISCUSSION

The role of transamination processes in growth and protein synthesis has not been unequivocally established. In general, in young parts of plants a high transamination activity was found and a direct correlation of these processes with protein synthesis (Albaum and Cohen 1943, Krietowich and Bundiel 1949, Cook 1959). Certain data, however, for animal and tumour tissues point to other connections. A lower transamination activity along with a higher protein synthesis were noted (data quoted by Raczyńska-Bojanowska 1960; Berezov 1961; Nakata et al. 1964).

Eberts, Burris and Riker (1954) found no correlation between growth inhibition of crown gall sunflower tissues by certain amino acids in a concentration of $10^{-3}M$ and the activity of the corresponding transaminases at various levels of these amino acids.

The results earlier reported (Chirek — parts I and II, in press) and the present ones point to a close relation between the investigated transamination processes and growth and protein synthesis in callus and tumour tissues of tobacco. In tumour tissue where the inhibitory effect of morphactin was relatively low and the changes in protein content and synthesis nonsignificant, the transaminase processes did not show any major changes.

In callus, tissue growth inhibition and lowered protein synthesis were associated with a considerable enhancement of the transamination processes as compared with those in the controls. In determination of the particular nitrogen compounds fractions (Chirek — part I, in press), a certain increase in the acid-soluble nitrogen content was noted in callus tissues treated with higher morphactin doses (20, 40 mg/dm³). Perhaps it is the enhanced transamination activity leading to the formation of larger amounts of the amino acids which are the main components of the acid-soluble nitrogen fractions, that is responsible for this increase.

On the basis of the earlier and present results it would seem that morphactin seriously disturbs nitrogen metabolism in tobacco callus tissues. It has been earlier discovered by Buchenauer and Grossmann (1969) that changes in the amino acid content in tomatoes treated with morphactin, and in protein content in wheat (Krishchenko et al. 1969) also indicate that morphactin affects nitrogen metabolism. This, however, does not prove true for tumour tissues which react weakly to morphactin.

In the case of tobacco callus tissues treated with morphactin, enhanced production of glutamic acid may, in the way of transamination, favour its further transformation to hydroxyprolin which is a specific constituent of inactive proteins of cell walls. Deposition of proteins of this type is suspected in tobacco callus tissues treated with morphactin (Chirek — part II, in press).

A comparison of control tumour and callus tissues of tobacco in respect to the transamination processes investigated reveals a higher enzyme activity in tumour tissue, with a balance shifted towards alanine production, whereas in callus tissue the shift is towards glutamic acid formation.

Foster and Weber (1969) determined the free amino acids level in normal and tumour tissues of tobacco cultured in vitro. They found a lower level of all amino acids with the exception of glutamic acid in normal tissue. Tumour tissues contained particularly much alanine, serine, proline, leucine and phenylalanine.

The results presented here concerning alanine transaminase activity agree in principle with the above quoted data. The reaction balance markedly shifted towards alanine production in tumour tissue explains higher content of this substance. In callus tissue the balance is shifted towards glutamic acid formation. In spite of a somewhat less intensive activity of this process as compared with that in tumour tissue, the glutamic acid level may be higher because glutamate decarboxylase activity (also regulating glutamic acid level) is here one half that in tumour tissue (unpublished data).

To sum up the results it may be affirmed that morphactin significantly affects nitrogen metabolism in callus tissue of tobacco. Growth inhibition of callus tissues subjected to the action of morphactin is correlated with changes in the intensity of transamination processes and protein synthesis. Other enzymes associated with nitrogen and protein metabolism should be further investigated to establish the step at which the normal metabolic equilibrium is disturbed in these tissues. The fact, however, that tumour tissue reacts much weaker to morphactin, indicates that the primary action of this substance may be exerted on hormonal regulation. Therefore, there the source of the metabolic disturbances observed should be sought. These disorders, as it seems, are secondary effects of morphactin activity.

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Fizjologiczne i biochemiczne efekty działania morfaktyny IT 3233 na tkanki — kalusową i tumorową Nicotiana tabacum L.

III. Aktywność procesów transaminacji katalizowanych przez aminotransferazę L-alanina: 2-oksoglutaran

Streszczenie

1. W ekstraktach tkanek — kalusowej i tumorowej tytoniu stwierdzono aktywną transaminazę alaninową. Wyższa aktywność enzymu cechuje tkankę tumorową. Równowaga reakcji jest przesunięta w kierunku wytwarzania alaniny w tkance tumorowej, a kwasu glutaminowego — w tkance kalusowej.

2. Morfaktyna zastosowana do hodowli tkanek wywołuje wyraźną stymulację aktywności enzymu w tkankach kalusowych, a tylko nieznaczną w tkankach tumorowych.

3. Ujemna korelacja pomiędzy aktywnością procesów transaminacji a wzrostem i syntezą białek w badanych tkankach traktowanych morfaktyną wskazuje na ich wzajemne powiązania oraz na zakłócenie metabolizmu azotowego w tkance kalusowej. Tkanka tumorowa wykazuje wyższą odporność na działanie morfaktyny.

Przypuszcza się, że zachwianie metabolizmu azotowego w tkance kalusowej może być wtórnym skutkiem działania morfaktyny na jej gospodarkę hormonalną.