Inhibition of Mitotic Activity in Root Tips of *Vicia faba* by the Antibiotic Cyanein

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Introduction

Cyanein is a new antibiotic isolated from *Penicillium cyaneum* (Betina *et al.* 1962a). It is active against some pathogenic and nonpathogenic fungi and possesses practically no antibacterial and antiprotozoal activity. It was found that cyanein has a strong cytostatic effect against human cells (strain HeLa) in vitro (Betina *et al.* 1962b).

We have observed that cyanein in the concentration of 100 µg/ml caused a 90 per cent inhibition of growth of root tips in *Allium cepa* (Betina *et al.* 1963). In this paper we are presenting results of the studies of the mitotic activity of cells in the meristem of *Vicia faba* in the presence of cyanein.

Materials and methods

Seeds of *Vicia faba* L. var. Zborovický were used in our studies. All experiments were done in the dark at the temperature of $25 \pm 0.2^\circ\text{C}$. Seeds were immersed into distilled water for 24 hours and then germinated in moistened sawdust for 60 hours. Seedlings with roots of a length of 4–5 cm were washed with distilled water and devoided of their plumules. The roots were put into small holes in a plate made of plexiglass which was placed onto a glass cubic dish so that roots were immersed into distilled water in the dish. Each dish contained 750 ml of distilled water and its lid from plexi-glass had holes for 23 roots. The content of the dish was continuously aerated. Roots were incubated in distilled water for 12 hours. After this period the lids with the roots in their holes were put onto other dishes, containing 750 ml of following solutions:

- a) Distilled water with cyanein of a concentration of 10 µg/ml.
- b) Distilled water with cyanein of a concentration of 50 µg/ml.
- c) 0.05% colchicine in distilled water containing cyanein in a concentration of 10 µg/ml.
- d) 0.05% colchicine in distilled water containing cyanein in a concentration of 50 µg/ml.
- e) Distilled water as a control for the solutions a and b.
- f) 0.05% colchicine in distilled water as a control for the solutions c and d.

During the incubation at $25 \pm 0.2^\circ\text{C}$ in the dark five to seven root tips were taken at the following intervals: 0, 1, 2, 4, 8, 12, 16, 20 and 24 hours. Root tips of a length of 1.5 mm were fixed and used for the preparation of the squashes (Murín 1960). Minimum 5,000 cells were scored for every fixation time. From these cells the mitotic index or accumulations of the c-metaphase during the first and the second mitotic cycle were determined.

Observations and explanations

Data about the individual phases of mitosis in meristematic cells of root tips of *Vicia faba* cultivated in water and in the solutions of cyanein are given in Table 1. Inhibitions of the mitotic activity by two concentrations of cyanein are presented graphically on Fig. 1. A decrease of the number of cells that are in mitosis is evident after 4 hours of the cultivation in water containing cyanein in concentrations of 50 $\mu\text{g/ml}$ and of 10 $\mu\text{g/ml}$, respectively.

Table 1. Phase of mitosis and mitotic indices in *Vicia faba* root tips in distilled water and in cyanein solutions

Time in hours	Phases per 1,000 cells				Mitotic index (in ‰)
	prophase	metaphase	anaphase	telophase	
control in distilled water					
0	51.1	16.3	12.3	9.7	89.4
1	49.1	16.3	13.0	14.1	92.5
2	47.0	13.4	15.9	13.5	89.8
4	51.7	12.4	12.5	10.4	87.0
8	49.8	19.8	16.7	16.0	102.3
12	47.9	14.0	9.6	12.4	83.9
24	33.4	13.1	9.9	11.4	69.8
cyanein 10 µg/ml					
1	44.7	15.7	12.4	11.0	83.8
2	51.6	16.7	11.9	9.8	90.0
4	26.1	11.1	8.9	9.4	55.5
8	38.4	12.6	7.6	10.3	68.9
12	19.8	9.8	7.6	4.6	41.8
24	4.0	2.1	1.6	1.0	8.7
cyanein 50 µg/ml					
1	51.4	13.9	11.3	13.9	90.5
2	49.6	11.1	11.9	14.8	87.4
4	22.6	5.4	5.6	7.7	40.9
8	1.2	1.4	0.6	0.8	4.0
12	0.5	0.3	0.0	0.2	1.0
24	1.0	0.7	0.3	0.1	2.1

The higher concentration of cyanein showed a very effective decrease of mitotic indices after 4, 8, 12 and 24 hours of cultivation. All phases of mitosis were inhibited and no structural disturbances of the mitotic apparatus were observed.

After 24 hours of cultivation in water containing cyanein in a concentration of 10 $\mu\text{g/ml}$, roots were further cultivated in water without cyanein, and 24 hours later the numbers of individual phases of mitosis and the

mitotic index were determined (see Table 2). It was found that this concentration of cyanein caused only a reversible inhibition.

The cultivation in water containing 0.05% colchicine and in the presence of cyanein in concentrations of 50 $\mu\text{g/ml}$ and of 10 $\mu\text{g/ml}$ enabled an observation of the accumulation of c-metaphases. Data on these observations are presented on Fig. 2.

Fig. 1. Frequencies of mitotic indices of *Vicia faba* root tip squashes. Each point represents the number of mitoses per 1,000 cells. Open circles, controls in distilled water. Filled circles, cyanein (10 $\mu\text{g/ml}$) in distilled water. Triangles, cyanein (50 $\mu\text{g/ml}$) in distilled water.

remained on a low level.

Cyanein in the concentration of 50 $\mu\text{g/ml}$ caused a decrease of the accumulation of c-metaphases. The second mitotic cycle did not start up to 24 hours of cultivation, while it started at 12 hours of the cultivation in the control medium, i.e. 0.05% colchicine.

Cyanein in the concentration of 10 $\mu\text{g/ml}$ in 0.05% colchicine also caused a decreased accumulation of c-metaphases and a prolongation of the first mitotic cycle. Even when the second mitotic cycle started at 12 hours of cultivation, the accumulation of tetraploid c-metaphases

Table 2. Mitoses in *Vicia faba* root tips without, during and after the treatment with cyanein

Time in hours	Cultivation*	Phases per 1,000 cells				Mitotic index (in ‰)
		prophase	metaphase	anaphase	telophase	
0	A	51.1	16.3	12.3	9.7	89.4
	A	33.4	13.1	9.9	11.4	69.8
24	B	4.0	2.1	1.6	1.0	8.7
	C	32.1	19.1	11.1	6.9	69.2

A-in distilled water (control)

B-in distilled water, containing cyanein 10 $\mu\text{g/ml}$

C-in distilled water, after the cultivation in B for 24 hours

Our observation can be concluded as follows: cyanein in the concentrations used inhibited the entrance of the cells into mitosis, which caused a prolongation of the first mitotic cycle at the lower concentration, i.e. 10 $\mu\text{g}/\text{ml}$. Because the second mitotic cycle started after 12 hours at this concentration of cyanein, it means that the reduplication of chromosomes in interphase was realized. The low level of the accumulation of tetraploid c-metaphases, evidently, is a result of the inhibition of the entrance of the cells into mitosis and of the prolongation of the first mitotic cycle.

Cyanein in the concentration of 50 $\mu\text{g}/\text{ml}$ in 0.05% colchicine caused a more efficient inhibition which made the second mitotic cycle impossible up to 24 hours.

Relative strong anti-mitotic effects of cyanein observed in our experiments with *Vicia faba* and the strong inhibition of the elongation of roots in *Allium cepa* (Betina *et al.* 1963) show some similarities of cyanein with other cytostatic antibiotics. Truhaut and Deysson (1960) studied antimitotic activity of mitomycin C on meristematic cells in *Allium sativum*. Mertz (1961) observed chromatic aberrations in lateral root tip chromosomes of *Vicia faba* treated with mitomycin C. Mattingly (1962) observed that actinomycin D inhibited the elongation of roots in *Vicia faba*. Bal and Gross (1963) published results of their detailed studies of mitosis and differentiation in roots of *Allium cepa* treated with actinomycin D. Actinomycin D in a concentration of 90 $\mu\text{g}/\text{ml}$ suppressed mitosis in root meristem and the differentiation of prevascular tissue. The block occurred at interphase. These authors showed actinomycin D inhibited synthesis of ribonucleic acid.

Fig. 2. Accumulation of c-metaphases in *Vicia faba* root tip squashes. Each point represents the number of c-metaphases per 1,000 cells. Full lines, controls in distilled water containing 0.05% colchicine; c-metaphases with 12 (open circles), 24 (half filled circles), and 48 chromosomes (filled circles). Dashed lines, cyanein (10 $\mu\text{g}/\text{ml}$) in same medium; c-metaphases with 12 (open squares), and 24 chromosomes (half filled squares). Dashed and dotted lines, cyanein (50 $\mu\text{g}/\text{ml}$) in same medium; c-metaphases with 12 chromosomes only (open triangles).

Summary and conclusions

In the presence of cyanein in the concentrations of 50 $\mu\text{g/ml}$ and of 10 $\mu\text{g/ml}$ mitosis in root meristem in *Vicia faba* ceases after 4 hours. The lower concentration of the antibiotic causes reversible effects. Cyanein in the concentration of 50 $\mu\text{g/ml}$ in water containing 0.05% colchicine causes a considerable decrease of c-metaphases after 2 hours and prevents the second mitotic cycle. The concentration of 10 $\mu\text{g/ml}$ causes a decrease of c-metaphases, a prolongation of the first mitotic cycle and a low level of tetraploid c-metaphases in the second mitotic cycle. Cyanein acts as a pre-prophase inhibitor.

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