ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. XXXVI — nr 4
1967

Action of 8-azaguanine and of inhibitors of protein synthesis on bud inducing activity of kinetin in the moss *Ceratodon purpureus*

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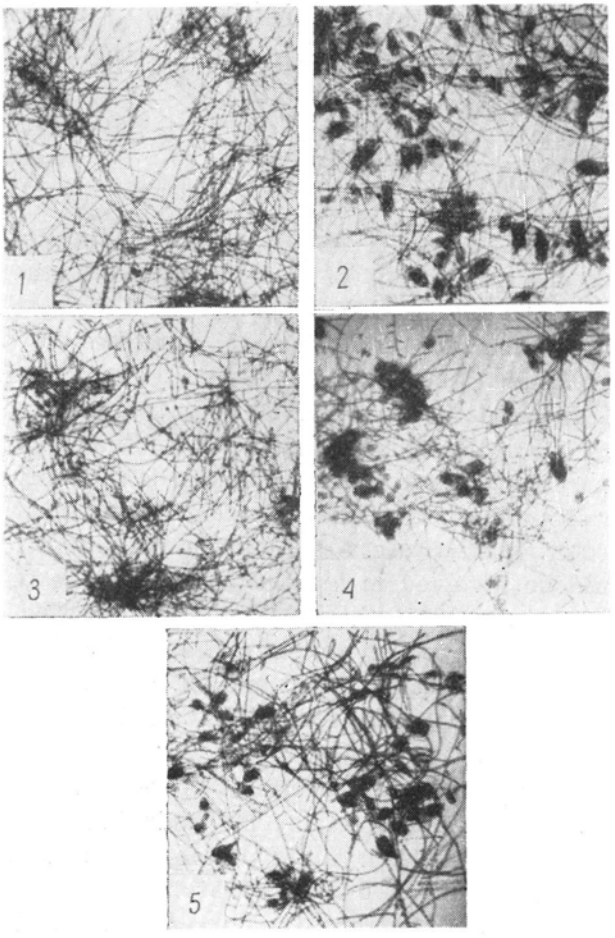
Cultures of the protonema of mosses in which the initiation of gametophore buds is highly influenced by kinetin constitute a useful morphogenetic system for investigating processes involved in the activity of cytokinins. As shown by Brandes and Bopp (1965) who obtained a complete inhibition of both spontaneous and kinetin-induced formation of gametophore buds with actinomycin D, the differentiation of gametophores in moss protonema is probably controlled by the activation of synthesis of DNA-dependent RNA, kinetin acting here as a gene effector. In this case, a stimulation of both RNA and protein synthesis should be involved in kinetin action. On the other hand, our previous investigations (1965) showed that bud induction in *Ceratodom purpureus* is not related to the protein concentration in the protonema. However, synthesis of specific RNA and of new specific proteins or enzymes might be responsible for both bud induction and kinetin activity in this process.

In a series of experiments, the effects of 8-azaguanine and specific inhibitors of protein synthesis on the bud-inducing activity of kinetin have been investigated. A 14-days old protonema samples of *Ceratodon purpureus* grown on liquid Kofler (1959) medium with the addition of 0.25 per cent glucose were transferred to an inorganic Kofler solution (basal control), to Kofler solution + 1 mg/l kinetin (kinetin control) and to Kofler solution + kinetin + inhibitor. The cultures were then observed during 10 days. In basal controls, no gametophore buds were formed during this time or occasionally only few buds appeared at about the tenth day (fig. 1). In kinetin controls, large number of buds appeared after two days (fig. 2).

The base analogue 8-azaguanine in concs. of 0.01—2.0 mM/l used as a possible inhibitor of RNA synthesis had very little effect on the bud-inducing kinetin action. In 1 mM/l, the buds were only slightly smaller and less numerous than in kinetin controls, and in 2 mM/l their appearance was additionally delayed for two days. The results are inconclusive, this, however, may be caused by generally low specificity of action of this base analogue (cf. Noodén and Thimann 1966).



1. Basal control; 2. Kinetin control; 3. Kinetin + fluorophenylalanine 0.1 mM; 4 — Kinetin + fluorophenylalanine 0.1 mM + phenylalanine 0.2 mM; 5 — Transferred from kinetin + fluorophenylalanine 0.1 mM to kinetin without the analogue.

As inhibitors of protein synthesis, chloramphenicol and three amino acid analogues, β-2-thienylalanine, m-fluorophenylalanine and 5-methyltryptophane were used in concs. of 0.01—5.0 mM/l. Chloramphenicol, thienylalanine and fluorophenylalanine at 0.1 mM, and 5-methylthryptophane at 0.5 mM brought about a complete inhibition of bud formafion without any apparent signs of toxicity (fig. 3). The inhibiting effect of thienylalanine could not be reversed either with alanine or by transferring the protonema to a medium without the analogue. On the other hand, phenylalanine at 0.2 mM included to the solution together with 0.1 mM of fluorophenylalanine prevented the inhibiting effect of the latter. The buds appeared after the same time as in kinetin control and their number was only slightly lower (fig. 4). Similarly, the inhibition by 0.5 mM of 5-methyltryptophane was largely prevented by 1 mM of tryptophane. With these two analogues, a reversal of inhibition was also obtained when after six days the protonema was transferred to a medium containing kinetin and no analogue (fig. 5).

Our previous investigations (1965) showed that the bud induction is not related to the protein concentration in the protonema and recently Brandes (1967) studying the uptake and distribution of ^{14}C-phenylalanine in the presence of kinetin found that the availability of protein-building material is not the limiting factor during kinetin-induced differentiation. The results presented in this communication, however, give an evidence for an involvement of protein synthesis in the kinetin effect on bud initiation in mosses, the synthesis of a specific protein (or proteins) probably being responsible for bud induction and kinetin activity.

This work was supported by a grant from the Botanical Committee of the Polish Academy of Sciences.

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(Entered: 15.VI.1967)

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Działanie 8-azaguaniny i inhibitorów syntezy białka na pączkotwórczą aktywność kinetyny u mchu Ceratodon purpureus

Streszczenie

Kinetyna jest bardzo aktywnym czynnikiem indukcji pączków gametoforowych w kulturze splątka mchów. 8-Azaguanina wpływa tylko nieznacznie hamująco na pączkowtórczą aktywność kinetyny, co jednak może być spowodowane stosunkowo małą specyficznością tego inhibitora. Natomiast inhibitory syntezy białka (chloramfenikol, β-2-tienyloalanina, m-fluorofenyloalanina, 5-metylotryptofan) w odpowiednich stężeniach blokują aktywność kinetyny w procesie pączkotwórczym, nie posiadając przy tym działania toksycznego, o czym świadczy odwracalność ich działania z pomocą odpowiednich aminokwasów lub po przeniesieniu do pożywki nie zawierającej inhibitora. Ponieważ z innych badań wynika, że efekt pączkotwórczy kinetyny nie jest zależny od intensywności syntezy białka, autorki wnioskują, iż synteza małych ilości specyficznego białka jest odpowiedzialna za różnicowanie gametoforów i za aktywność kinetyny w tym procesie.