THE INFLUENCE OF β-INDOLEACETIC ACID AND GIBBERELLIN ON THE TEMPLATE ACTIVITY OF THE CHROMATIN OF WHEAT COLEOPTILES OF DIFFERENT AGES

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1. Introduction

At the present time, the mechanism of the action of phytohormones is a matter of a wide discussion [1–3]. The hypothesis that the phytohormones, just as animal hormones, are genetic inductors activating selectively the DNA-dependent RNA and protein synthesis in hormone sensitive tissues is the most interesting one. But experimental data concerning this problem are rather contradictive [5, 6]. According to our previous studies the influence of phytohormones is closely connected with the age of cell [7–9]. That is why it was reasonable to elucidata the influence of β-indoleacetic acid (IAA) and gibberellin (GA_3) on the chromatin-directed RNA synthesis in wheat coleoptiles.

2. Materials and methods

Wheat coleoptiles (*Triticum vulgare*, spring) of different ages have been studied. Wheat seeds were washed with tap water. Then they were soaked in distilled water at +25°C for 2 hr. Seeds were germinated on a moist sterile filter paper in large Petri dishes. The Petri dishes were then kept in a thermostat at +25°C. The coleoptiles were separated by hand 36, 72 and 120 hr after soaking. These periods coincide correspondingly with cell division (36 hr), with the middle of the phase of 'pure extensibility' of the cell (72 hr) and with the beginning of the aging (120 hr) of the coleoptile cells [10]. The treatment with phytohormones was performed as follows: After 2 hr soaking in water, seeds were germinated on a solution with GA_3 (15 mg/l) during 36, 72 and 120 hr. The seedlings were transferred to the solution with IAA (0.5 mg/l) 12 hr before the separation of the coleoptiles. The control seedlings were germinated on distilled water. Chromatin was isolated from the tissue according to the method of Zubay and Doty [11] with following modifications. The tissue was homogenized in a Potter–Elvehjem blender with a driving motor for 3 min at 2500 rev/min in a grinding medium containing 15% glycerol in a standard saline solution (0.075 M NaCl, 0.024 M EDTA pH 6.0). All the procedures were carried out in a cold room at +2°C. The homogenate was filtered through 2 layers of a nylon cloth to remove cell wall debris. The remainder on the nylon cloth was homogenized twice in a grinding medium. The filtrate was then centrifuged for 25 min at 3500 *g*. The pellet was resuspended in a standard saline solution and sedimented for 15 min at 3500 *g*. This procedure was repeated 6 times. After the last centrifugation the pellet was homogenized in 0.14 M NaCl and in 0.01 M Tris–HCl buffer pH 8.0 twiced and centrifuged for 15 min at 3500 *g*. The chromatin pellet was homogenized in 1.4 M sucrose on 0.01 M Tris–HCl buffer pH 8.0, and layered on a 6-fold volume of 1.4 M sucrose on 0.01 M Tris–HCl buffer pH 8.0. The tubes were centrifuged for 1.5 hr at 30 000 *g*. The pellet was dissolved in bidistilled water and clarified at 4000 *g* for 40 min. The DNA content of the chromatin preparation was measured according to Dische [12] and by absorbance at 260 nm. The separation of RNA and DNA was performed according to Schmidt and Tannhauser

[13] with subsequent determination according to Springer [14]. Protein was determined by the method of Lowry et al. [15]. Chromatin entirely corresponded to the standards of purity according to Bonner [16]. Template activity of the chromatin was determined as described previously [17]. In each variant the preparation of chromatin was carried out 2–4 times, and the determination of RNA-synthesizing ability of chromatin 4–6 times. The results were statistically treated. The mean square deviation was determined [18].

3. Results and discussion

It has been established that RNA synthesis is really occurring on the template of chromatin, isolated from wheat shoots, since the reaction depends completely on the presence of Mg^{2+} and Mn^{2+} in the incubation medium, and is inhibited by actinomycin D, RNase and rifamycin (table 1). Template activity of chromatin preparations becomes decreased with aging

Table 1

Influence of different inhibitors of RNA synthesis on the template activity of wheat chromatin

System	Incorporation [^{14}C] AMP in RNA (% of control)
1. Complete system	100
2. + Actinomycin D 12.5 μg	6.2
3. + Rifamycin 7 μg	0
4. + RNA-ase 50 μg	0
5. The system without Me^{2+}	9.6

The complete system contained the following components: 40 μmoles Tris–HCl pH 7.9; 2.5 μmoles β-mercaptoethanol; 2.0 μmoles $MgCl_2$ 0.5 μmoles $MnCl_2$; 0.2 μmoles each of CTP, UTP, GTP and ATP; 0.045–0.09 μC (^{14}C-ATP (specific activity 38 mCl/mmole, Amersham), chromatin equivalent to 2–40 μg of DNA, RNA-polymerase (Novosibirsk, SKTB BAV) – 10–30 units activity in a final volume of 0.5 ml. The assay was routinely run for 30 min at 37°C. The reaction was stopped by the addition of 0.1 ml 1% RNA and of 5 ml cold 5% trichloroacetic acid (TCA). TCA-insoluble product was recovered on Millipore filters Aufs (ČSSR), washed with 30 ml cold TCA and with 10 ml alcohol, dried and counted in a toluene scintillation fluid on the counter Mark 1 (Nuclear Chicago). The scintillation fluid consisted of toluene –1 l, PPO – 4 g, POPOP – 0.2 g.

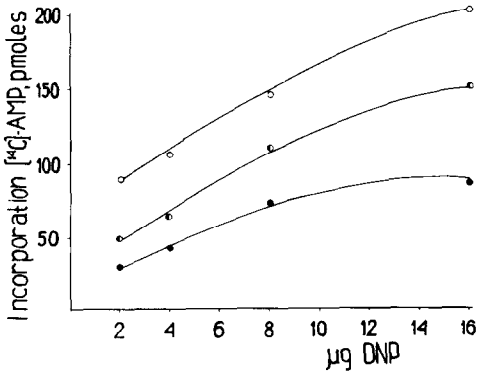

Fig. 1. Change of the template activity of chromatin in cells of different age of wheat coleoptiles. (○—○—○) – 36 hr; (◐—◐—◐) – 72-hr; (●—●—●) – 120 hr.

of the cells (fig. 1). Chromatin isolated from cells at the end of the phase of cell-division (36 hr) had the maximum template activity. Chromatin template activity of 72-hour seedlings is slightly reduced, and in 120-hr coleoptiles it is significantly reduced. If the chromatin template activity in 36-hr seedlings is taken as 100%, then in 72-hr one it is 76.3% ± 2.7, and 120-hr ones –45.6% ± 9.17. When the seedlings are germinated on GA_3 it results in a sharp increase of the template activity of chromatin, prepared from 36-hr coleoptiles. It amounts to 136.2 ± 3.9 in comparison with the control (fig. 2 a).

It is worth emphasizing that in seedlings of this phase has been observed previously established [7, 8]

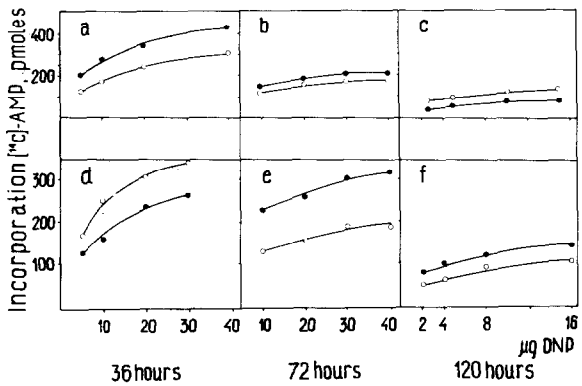

Fig. 2. Influence of the phytohormones on the template activity of chromatin isolated from wheat coleoptiles of different age. (○—○—○) – Control; (●—●—●) – with the phytohormones: a,b,c – GA (gibberellic acid); d,e,f – IAA (indolieacetic acid).

stimulates most strongly the growth of coleoptiles. However, as seen from fig. 2 b, the influence of GA_3 did not alter the template activity of chromatin isolated from 72-hr seedlings. Template activity of the chromatin isolated from 120-hr seedlings germinated on GA_3 exhibits a tendency to decrease against the control (fig. 2 c). This may be explained, probably, by the fact that GA_3 accelerates the aging of the coleoptile cells.

Fig. 2 d shows that germination of 36-hr seedlings on IAA solution inhibited the template activity of the chromatin by 23.8% ± 4.02 in comparison with the control. We have found earlier [7–9] that at this age under the effect of IAA the respiration rate, oxidative phosphorylation, activity of glycolytic and hexosemonophosphate enzymes, the processes of growth of the seedlings are inhibited. On the contrary, the germination of 72-hr seedlings on IAA solution results in the increase of the template activity of chromatin by 150.9% ± 6.1 against the control (fig. 2 e). The data concerning the stimulation of template activity of the chromatin under the effect of auxin 2,4D are available [5]. The increased template activity of chromatin isolated from 72-hr coleoptiles germinated on IAA, correlated with the increase of the respiration rate, the activity of glycolytic and hexosemonophosphate pathway enzymes, the activation of the process of the oxidative phosphorylation, and the expansion of the height of the seedlings [7–9]. The template activity of chromatin isolated from 120-hr seedlings germinated on IAA became reduced in comparison with that of 72-hr seedlings (fig. 2 f). No change in endogenous RNA-polymerase activity of chromatin under phytohormone treatment was observed in any variant.

These data show that IAA and GA_3 appear to have different effects on the changes of the template activity of chromatin in the cells of different ages. One may suppose that just these changes are the basis of the change of activity of a number of enzymes taking part in the process of respiration. It may be assumed that the cause of different responses of the template activity of chromatin isolated from the cells at different ages to the effect of exogenous auxin and GA_3, is connected with the fact that sensitivity to the phytohormones depends on the competence of the tissue. The competence of the tissue may depend on the presence of acceptors for a given phytohormone at a certain phase of cell growth.

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