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CYTOKININ-INDUCED CHANGES IN THE SPECTRA OF THE PROTEINS SYNTHESIZED IN COTTON-PLANT CHROMATIN

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The influence of cytokinin and the cytokinin-binding protein on the kinetics of the processes of transcription and translation in isolated nuclear chromatin from cotton-plant seedlings has been investigated. It has been shown by HPLC and electrophoresis in PAAG containing SDS that the phytohormone, in combination with the hormone-binding protein, induces qualitative and quantitative changes in the primary translation products.

One of the factors in the activation of transcription in plants is the formation of complexes of phytohormones with hormone-binding proteins. It is assumed that these complexes include specific genes that are transcribed into mRNA, which, in its turn, is translated into specific proteins [1, 2]. However, in the experiments that have been described whole plants or isolated organs were used. In view of this, the changes observed in the spectra of the proteins could be a consequence of other, earlier reactions of the cells to the phytohormones and not of their primary interactions with the cell genome. It therefore appeared desirable to investigate the direct action of cytokinins on protein synthesis in a model system containing nuclear chromatin, in which, as is known, are localized the associates of protein kinase with RNA polymerase that many authors regard as a possible target of phytohormones [3, 4].

In the present paper we give the results of a study of protein biosynthesis in the chromatin from cotton-plant seedlings and of the influence on this process of a number of inhibitors and activators of transcription and translation (Table 1).

As activators we used synthetic cytokinin 6-benzylaminopurine (BAP) and the site of the cytokinin-binding proteins (CBPs) isolated from cotton-plant seedlings. The results obtained confirmed those already available showing that plant chromatin is capable of incorporating labeled amino acids in the TCAA-precipitated fractions [5]. Some workers assume the formation of polypeptides by the polymerization of the amino acids on the chromatin DNA [6], while others consider that a posttranslational modification of already existing polypeptides by various products, including amino acids, takes place [7]. In our opinion, a third idea is more likely, according to which there is autonomous biosynthesis on hypothetical nuclear ribosomes [8]. In particular, such proteins could be multienzyme components of DNA replication, but the formation of proteins with a different function is not excluded.

Contamination of the chromatin with cytoplasmic ribosomes can be excluded, since this process is insensitive to puromycin and cycloheximide. At the same time, the modulation of protein synthesis by cytokinin and its complex with the CBP shows that both transcriptional and translational processes occur in isolated nuclear chromatin. These processes are closely linked. This is shown by the fact that an intensive incorporation of labeled markers — [^{33}P]-ATP and -RNA and [^{35}S]-methionine — into the TCAA-precipitable material begins simultaneously with the first few minutes of incubation (Fig. 1).

The addition of rifampicin — a specific inhibitor of RNA polymerase — completely suppressed the synthesis of mRNA (Fig. 1d) and appreciably suppressed the synthesis of protein (Fig. 1c), which again confirmed the conjugated nature of transcription and translation in isolated chromatin. The TCAA-precipitated proteins were then separated by reversed-phase liquid chromatography (Fig. 2). As compared with the control (Fig. 2a), in the samples with added BAP (Fig. 2b) and CBP (Fig. 2c) there was, judging from the size of the peaks, an increase in protein synthesis. It must be mentioned that the nature of the distribution of radioactivity coincides with the elution of the protein peaks. In the case where BAP was added to the complex with the CBP (Fig. 2d), however, two new fractions appeared (Fig. 2d-3 and d-4).

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TABLE 1. Influence of Transcription and Translation Activators and Inhibitors on the Inclusion of [^{35}S]-Methionine in the Chromatin Proteins Precipitated by Trichloroacetic Acid

Preparations	Concentration	Inclusion of [^{35}S]-Methionine, pulses/min	Inclusion, %
Control		190000	100
BAP	0,005 mM	222300	117
CBP	0,5 nM	228000	120
BAP+CBP	0,005 мМ+0,5 nM	264100	139
Puromycin	0,05 mM	190000	100
Cycloheximide	0,5 mM	188100	99
Rifampicin	0.05 mM	72200	38

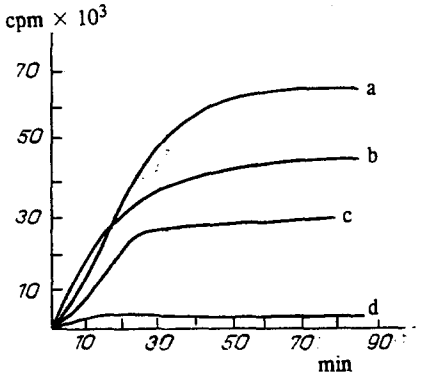

Fig. 1. Kinetics of the inclusion of [^{35}S]-methionine in polypeptides (a) and of [^{33}P]-ATP in RNA (b) in isolated chromatin, and the influence of rifampicin on this process (c, d).

The results obtained were confirmed by electrophoretic analysis in PAAG containing SDSNa (Fig. 3). It can be seen on the electrophoretogram that, as compared with the control, in the experiment with the BAP–CBP complex three protein bands had appeared, with molecular masses of from 14 to 20 kDa. It may be assumed that the polypeptides were present in fractions 2d-3 and 2d-4.

Thus, we have succeeded in showing that cytokinin in a complex with CBP activates processes of transcription–translation and induces qualitative and quantitative changes in the spectrum of the polypeptide products being synthesized. A further study of the structural–functional features of the polypeptides found will permit the elucidation of some aspects of the cytokinin-dependent regulation of gene expression and the mechanism of its realization.

EXPERIMENTAL

Plant Material. Seeds of a cotton plant of variety 48-80 were treated with concentrated sulfuric acid and were then washed with a large volume of main water and were steeped for 24 h. The seeds were then allowed to germinate between moist sheets of filter paper in a thermostat at 30°C for two days.

Isolation of the Chromatin. The seedlings were weighed and were treated with 5 volumes of a buffer containing 0.1 M Tris-HCl, pH 7.8, 0.25 M sucrose, 0.1 M $MgCl_2$, and 0.02 M mercaptoethanol and were homogenized at 14,000 rpm for 1.5 min. The homogenate was filtered through a layer of coarse calico and gauze and was centrifuged at 1300g for 10 min. The deposit was suspended in a buffer containing 0.01 M Tris-HCl, pH 7.8, 0.35 M sucrose, 0.01 M 2-mercaptoethanol, and 2% of Triton X-100 and was centrifuged at 7000g for 2 min. This procedure was repeated 3 times, after which the deposit was washed twice with the same buffer without the Triton. The final chromatin residue was suspended in a buffer of the following composition: 0.05 M Tris-HCl, pH 7.8, 0.002 M $MgCl_2$, 0.001 M 2-mercaptoethanol, and 10% of glycerol, and was used for the experiments [9].

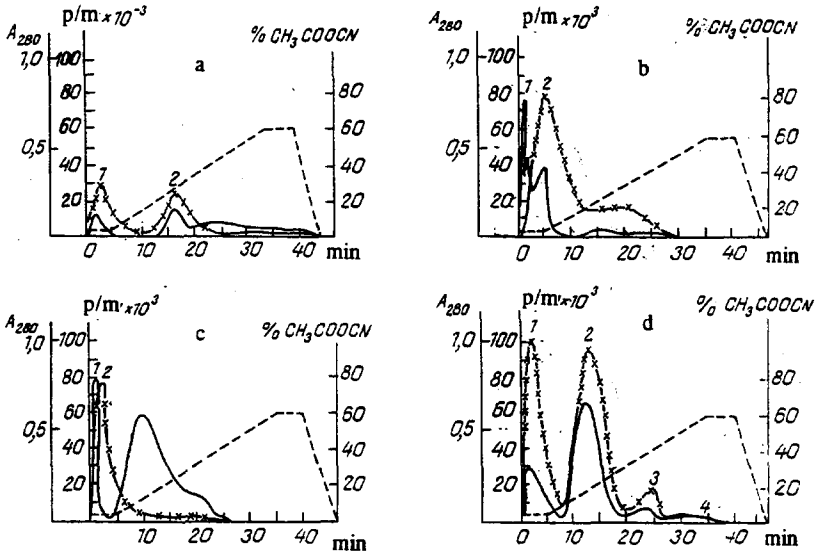

Fig. 2. HPLC analysis of the polypeptide products synthesized in isolated chromatin in the presence of [^{35}S]-methionine: a) control; b) BAP; c) CBP; d) BAP+CBP [—) percentage of acetonitrile; – –) protein absorption; ***) radioactivity].

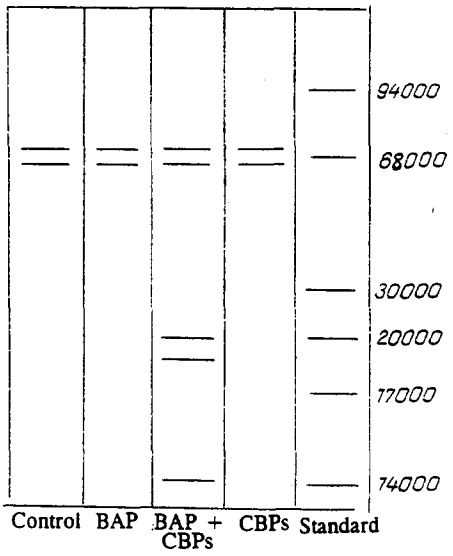

Fig. 3. Electrophoresis in PAAG of the translation products precipitated in trichloroacetic acid.

Determination of the Transcription of the Chromatin. In these experiments we used a reaction mixture with the following compostion: 50 mM Tris-HCl, pH 7.8, 8 μM $MgCl_2$, and 0.1 μM each of ATP, UTP, GTP, and CTP. As the labeled precursor we used [α-^{33}P]-ATP (produced by the Radiopreparat Experimental Factory, Tashkent, molar activity 37 PBq/mole) in an amount of 25 μl. To 0.25 ml of the reaction mixture, was added 0.1 ml of a chromatin suspension containing 1.0 μg of DNA, and the sample was incubated at 30°C for 25 min. The reaction was stopped by rapid cooling in ice, and by the addition of 1 ml of 10% TCAA. After 30 min, the sanple was deposited on Synpor filters (Czechoslovakia) and was washed 3 times with 5% TCAA and 5 times with 96% ethanol. After this, the radioactivity of the acid-insoluble residue was determined in ZhS-106 scintillation liquid in a Betta-1 counter.

Translation of mRNA. The same conditions were used in the system for translation as in the determination of transcription. As the labeled precursor, 50 μl of [^{35}S]-methionine (produced by the Radiopreparat Experimental Factory, Tashkent, molar activity 12.5 PBq/mole) with a total activity of 1 MBq was added.

Isolation of the Total Labeled Proteins of the Chromatin. After incubation, the proteins precipitated by 10% TCAA were washed free of unbound radioactivity, suspended in ethanol, and centrifuged at 7000g for 10 min. This procedure was repeated until radioactivity had disappeared from the supernatant. The residue was suspended in 0.5% ammonia solution with 5% of SDS and was centrifuged at 7000g for 30 min. This procedure was repeated 5 times and the combined supernatants were dialyzed against water and were then lyophilized.

The translation products were analyzed by electrophoresis in PAAG in the presence of Na-DDS according to Laemmli [10].

DNA contents were determined colorimetrically [11].

The HPLC analysis of the reaction products was conducted on a Du Pont 8800 instrument (USA) using an Ultropack TSK TMS-250 column with dimensions of 4.6 × 75 mm in 0.1% trifluoroacetic acid with an acetonitrile gradient of from 5 to 60%. The time of separation was 40 min, and the sensitivity 0.64 O.U. at 280 nm. Rate of flow 0.5 ml/min, chart speed 30 cm/h, volume deposited 100 μl.

The isolation of the CBP site was done on three-day cotton-plant seedlings by affinity chromatography on BAP-acetamidohexyl-Sepharose 4B by a method described previously [12].

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