Chemistry of Natural Compounds, Vol. 33, No. 6, 1997

INFLUENCE OF THE GLYCOPROTEINS OF THE COTTON PLANT ON THE BIOSYNTHESIS OF PROTEINS AND NUCLEIC ACIDS

Z. S. Khashimova, O. Kh. Saitmuratova, Yu. S. Mangutova, and V. B. Leont'ev

UDC 612.085.4:577.12.3.853

An inhibitory action of ELPs and LLPs on the inclusion of ^{3}H-thymine and ^{35}S-methionine in the cells of a suspension culture and isolated nuclei of cottonplant seedlings has been found. The influence of the ELPs on the proliferative activity of cottonplant cells and of the ELPs and LLPs at the level of protein biosynthesis in isolated nuclei bears a dose-dependent nature, in contrast to the galactose-specific fraction LLP-1.

Interest in plant glycoproteins is due to the diversity of their functions in the plant itself and also to the broad spectrum of their biological activity. The participation of glycoproteins, including lectins and lectin-like proteins (LLPs), in the aggregation and adhesion of normal and tumor cells [1] and in the agglutination of blood cells [2], and their mitogenic and cytotoxic properties, connected with the action of these proteins both at the membrane level and at intracellular levels [3, 4], are known. Many lectin and lectin-like proteins act on the biosynthesis of proteins [5].

The use of cell-free systems for the biosynthesis of the most important cellular biopolymers — nucleic acids and proteins — enables the biological activity of new protein substance to be evaluated.

In this connection, we have studied the action of LLPs and extensin-like proteins (ELPs) on the level of proliferation of cottonplant cells and on the biosynthesis of proteins in isolated nuclei.

We used the total lectin-like proteins from cotton seeds and also the galactose-specific LLPs (LLPs-1) obtained from the total LLP fraction by affinity chromatography on a column of Sepharose 4B (Fig. 1). Electrophoretic analysis of the LLPs was carried out in 15% PAAG with 0.1% of sodium dodecyl sulfate (Fig. 2*a*, *b*).

The extensin-like proteins (ELPs) were isolated from a suspension culture of the cotton plant and were characterized electrophoretically in 7.5% PAAG in the presence of 8 M urea (Fig. 3, *a*, *b*, *c*). The presence of the glyco fragment was shown by treating the gel with Con-A peroxidase, followed by staining with diaminobenzidine. We had previously established the presence of carbohydrates in the lectin-like proteins [6]. In the ELPs, as can be seen from Fig. 3, *c*, there were two carbohydrate-containing zones, which showed that these substances were glycoproteins.

Analysis of the composition of the glycoproteins by Lowry's method for the protein part and with the use of the anthrone–acid reagent to determine the total sugars showed 11.6 and 18.0% of carbohydrates in the LLPs and the LLPs-1, respectively; for the ELPs we determined the ratio of protein and carbohydrates as 1:4.

We had previously established the influence of the lectin- and extensin-like proteins of the cotton plant on the proliferative activity of cells of various types: lymphocytes, myelocytes, and K-562 human erythroblasts (by evaluating the levels of DNA synthesis) [7].

In the present paper we give the results of a study of the proliferative activity of the cells in a suspension culture of the cotton plant by determining the inclusion of ^{3}H-thymine. For this, the cells were sown in an amount of about 100 thousand cells per well, and the proteins were added to the culture medium in doses of from 10 to 100 μg per well for 24 h. As can be seen from Table 1, the LLPs and ELPs inhibited the inclusion of ^{3}H-thymidine in the DNA of cottonplant cells, the proliferative activity of the cells falling substantially even at a dose of ELPs of 25 μg (55.1%) and scarcely changing when the dose was increased to 100 μg. The LLPs exhibited an analogous effect at a dose of 100 μg.

A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 62 70 71. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 865-870, November-December, 1997. Original article submitted July 1, 1997.

 0009-3130/97/3306-0676$18.00

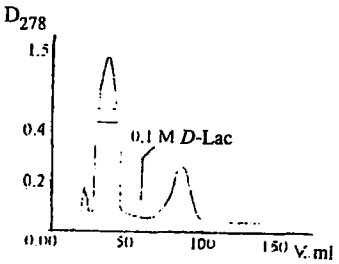

Fig. 1. Affinity chromatography of the lectin-like proteins from cotton seeds on a 1.5 × 24 cm column of Sepharose 4B in 0.14 M NaCl−0.01 M Na phosphate buffer, pH 7.4. Rate of elution 12 ml/h.

Fig. 2. Electrophoresis of the LLPs in 15% PAAG in the presence of 0.1% of sodium dodecyl sulfate: *a*) total LLP fraction (LLPs 60-80); *b*) LLPs-1.

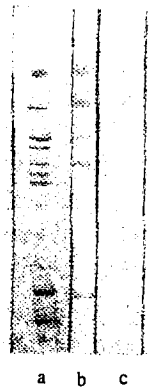

Fig. 3. Electrophoresis of the ELPs in 7.5% PAAG in the presence of 8 M urea: *a*) total; *b*) ELPs obtained after purification on a CM-cellulose column (staining in Coomassie Blue); *c*) ELPs (gel stained with diaminobenzidine).

It is interesting to note that concanavalin A (Con-A), known as an extremely powerful mitogen of animal and human lymphocytes but toxic for some types of animal cells [1], inhibited the inclusion of the label in DNA in cottonplant cells to the same degree as the ELPs but at a lower dose (10 μg); i.e., it exhibits a powerful inhibitory action on the biosynthesis of DNA in vegetative cells of the cotton plant. The same type of action of the ELPs isolated from a suspension culture of the cotton

TABLE 1. Action of Lectin- and Extensin-like Proteins on the Proliferation of Cottonplant Cells

Sample	Percentage inclusion of ^{3}H-thymine at the given doses of protein in the sample, μg		
	10	25	100
Control	100.0	100.0	100.0
LLPs	–	–	68.9
ELPs	92.6	55.1	58.4
Con-A	51.5	–	–

TABLE 2. Action of Lectin- and Extensin-like Proteins of the Cotton Plant on the Biosynthesis of Proteins in Isolated Nuclei

Sample	Percentage inclusion of ^{35}S-methionine at the given doses of protein in the sample, μg			
	10	50	100	200
Control	100.0	100.0	100.0	100.0
LLPs	–	73.0	53.0	35.9
LLPs-1	–	59.5	–	54.5
ELPs	74.0	30.7	19.3	–

plant was shown in relation to animal myeloma cells and K-562 cells: at low doses the level of inclusion of thymidine was close to that of a control, but with an increase in the dose the degree of inhibition rose sharply [7].

On the other hand, the influence of the lectin-like proteins in different cell systems bears a contrary nature. Thus, while in human cells (lymphocytes) and animal cells (mouse myelocytes) these proteins exhibit mitogenic properties, in a homologous system (suspension culture of the cotton plant) they lead to an inhibition of the synthesis of DNA. These differences are apparently due to the specific nature of the interaction of the ELPs and the LLPs with the cell receptors of homologous and heterologous systems.

It is known that the mitogenic stimulation of lymphoid cells under the action of lectins includes binding with the carbohydrate components of the cell receptors acting as "triggers" initiating a cascade of intracellular processes, including intranuclear ones [8]. Therefore, it is possible to determine the nature of the action of lectin and lectin-like proteins by evaluating the level of synthesis of nucleic acids and proteins in the nucleus.

In this connection, we have investigated the influence of the LLPs and ELPs that we had isolated on the biosynthesis of proteins in isolated nuclei *in vitro*. We isolated the nuclei from two-day cottonplant seedlings. The purity of the nuclei obtained was checked under the microscope.

We had previously studied features of the biosynthesis of protein in the nuclei of animal and plant cells and had determined the composition of the incubation medium and the optimum regime for evaluating the biosynthetic activity of cottonplant nuclei [9]. We used these experimental conditions in the present work. In order to study the biosynthesis of protein in isolated nuclei, to a reaction mixture containing 0.5 ml of a nuclear suspension (1 mg/ml) we added LLPs, LLPs-1, and ELPs in doses of from 10 to 200 μg per sample (Table 2). We used ^{35}S-methionine as the radioactive precursor.

As can be seen from Table 2, the LLPs and ELPs substantially lowered the level of inclusion of the label in the isolated nuclei, the influence of the ELPs being shown more strongly and having a dose-dependent nature. The inhibitory properties of the galactose-specific proteins (LLPs-1) were shown at a dose of 50 μg, and this effect scarcely changed at a dose of 200 μg. The total LLPs, in contrast to the LLPs-1, include proteins with various carbohydrate specificities, and we therefore assume that the higher dose of LLPs is due to the number and specific nature of the recognition sites on the surface of the nuclear membrane.

In connection with this hypothesis, we have studied the influence of the ELPs on the template activity of isolated chromatin (from the inclusion of ^{3}H-CTP). At protein doses of 25, 50, and 100 μg, the inclusion of ^{3}H-CTP amounted to 4284, 5343, and 3757 pulses per minute (ppm), respectively, at a control figure of 2812 ppm. The chromatin was isolated from two-day cottonplant seedlings. (This section of the work was performed in collaboration with the Laboratory of Protein and Peptide Chemistry.)

Thus, it was established that the ELPs activated the template synthesis of nucleic acids by a factor of 1.5-2. In the nature of the action of the ELPs, this effect is the direct opposite of their inhibiting influence on the synthesis of nucleic acids and proteins in intact cells and nuclei. Apparently, the absence of membrane receptors provides the possibility of a direct action of the ELPs on the chromatin or its components, with a change in template activity. The observed increase

in the level of nucleic acid synthesis on a chromatin template is probably connected with processes of the decompactization of the chromatin followed by its activation.

The experiments with cell-free systems showed that both groups of cottonplant proteins — ELPs and LLPs — are inhibitors of processes involved in the biosynthesis of nucleic acids and proteins in cells and in isolated nuclei, respectively. This conclusion is in harmony with known facts on the cytotoxicity of plant lectins. Among the latter, in addition to ricin and similar substances, lectins have been isolated in a group of so-called ribosome-inactivating proteins from the seeds of maize, wheat, rye, barley, and other crops. Some of them (gelonin, bryodin) are not toxic for intact cells but suppress the ribosomal synthesis of proteins in cell-free systems. These proteins have been shown to possess antiviral, fungicidal, and other types of activity, which impart to them the nature of protective proteins [10, 11]. Clearly, while recognizing a difference in the mechanisms of biosynthesis on ribosomes and in isolated nuclei, possibly with the participation of multienzyme complexes and template DNA [12], we may note that the general tendency to the manifestation of the biological activity of cottonplant lectins as inhibitors of the biosynthesis of proteins is retained. In the final account, of fundamental importance is the very fact of the active influence of cottonplant glycoproteins at the level of the genetic apparatus in the cell nucleus.

EXPERIMENTAL

The lectin-like proteins were isolated from seeds of a cotton plant of the 108-F variety. The seeds were freed from skin, finely ground in a mortar, treated with a cold 1:1 mixture of acetone and ether, and dried in a vacuum desiccator. The proteins were extracted from the seed flour so obtained by two treatments with a 0.14 M NaCl solution, pH 3.2, containing 1 mM PMSF as a protease inhibitor, 1 mM ascorbic acid to prevent the oxidation of phenols, and 0.02 mg/ml of sodium bisulfate to bind low-molecular-mass polyphenols, in which cotton seeds are extremely rich [13]. The supernatants collected after centrifugation were subjected to stepwise salting-out with ammonium sulfate at 30, 60, and 80% saturation [14]. We used the 60-80% fraction of LLPs, which we designated as the total LLP fraction.

To isolate the galactose-specific proteins, we subjected the 60-80% LLPs to affinity chromatography on Sepharose 4B (Pharmacia) in a 1.5 × 24 [cm] column equilibrated with 0.14 M NaCl in 0.01 M Na phosphate buffer, pH 7.4. For binding, the LLPs were preincubated on the column for 1 h and were then washed with the working buffer at a rate of elution of 12-14 ml/h. The LLPs-1 were desorbed with 0.1 M *D*-galactose in the same buffer. To detect proteins we used a Uvicord (LKB, Sweden) with 226 nm and 278 nm filters.

The proteins obtained were characterized electrophoretically in 15% PAAG in the presence of 0.1% of sodium dodecyl sulfate [15].

The extensin-like proteins were isolated from a suspension culture of cottonplant cells by extraction with 0.2 M $CaCl_2$ and were purified on a column of CM-cellulose. The proteins obtained were characterized electrophoretically in the 7.5% PAAG/8 M urea system.

Nuclei were isolated from two-day cottonplant seedlings. For this purpose, cotton seeds were treated with concentrated sulfuric acid to eliminate fibers and were washed with water to a neutral pH. The seeds treated in this way were germinated in rolls of moist filter paper for two days at 28°C. The seedlings were collected and were homogenized in solution 1 (10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, O.1 M PMSF) containing 0.25 M sucrose. The homogenate was filtered through two layers of gauze and was centrifuged at 600 rpm for 5 min (K-23). The deposit was discarded, and the supernatant was centrifuged at 3.5-4 thousand rpm for 15 min. The resulting deposit of nuclei was suspended in solution 1 containing 1.8 M sucrose, and the suspension was layered on the same buffer containing 1.8 M sucrose in centrifuge tubes from a SW-28 rotor (Beckman) and was centrifuged at 100,000 *g* for 90 min. The nuclear deposit was suspended in solution 1 and centrifuged at 4000 rpm for 15 min. The nuclei so purified were used for further work.

All the biochemical operations were conducted at 4°C.

The suspension culture of cottonplant cells was kindly provided by I. Grigina (Institute of Genetics, Academy of Sciences of the Republic of Uzbekistan).

Cells were incubated with the preparation at 28°C for 24 h. Four hours before the end of cultivation, Amersham ^3H-thymidine was added in an amount of 10 μCi per well in a volume of 10 μl.

To evaluate the level of inclusion of radioactivity, 300 μl of resuspended cell suspension was taken from each well and was transferred to GFC filters, fixed with 5% TCA, washed with distilled water, dried with alcohol, and counted in 5 ml of scintillator (PPO-POPOP in toluene) in a liquid scintillation counter.

All the experiments, including the controls, were performed in triplicate.

The hemagglutinating activity of the LLPs was determined with the use of a 2% suspension of human blood by a standard method.

The levels of total sugars in the proteins were determined spectrophotometrically with the anthrone–sulfuric acid reagent.

Proteins were determined by Lowry's method [16].

REFERENCES

1. N. Sharon and H. Lis, Lectins, Chapman and Hall, London (1989), p. 26.
2. D. Ashford, R. A. Dwek, N. Sharon, and T. Rademacher, Eur. J. Biochem., **311**, 166, 320 (1987).
3. V. M. Lakhtin, Biotekhnologiya, No. 6, 676 (1989).
4. I. M. Lord, Plant Physiol., **85**, 1 (1987).
5. F. Stirpe, L. Barbiere, and A. Abbondanza, J. Biol. Chem., **260**, 14589 (1985).
6. Yu. S. Mangutova, Z. S. Khashimova, D. M. Katalaeva, M. É. Suslo, and V. B. Leont'ev, Dokl. Akad. Nauk RUz, No. 3, 40 (1989).
7. Z. S. Khashimova, Yu. S. Mangutova, M. É. Suslo, D. M. Beknazarova, and V. B. Leont'ev, Khim. Prir. Soedin., 912 (1996).
8. H. R. MacDonald and M. Nabholz, Ann. Rev. Cell Biol., **2**, 231 (1986).
9. O. Kh. Saitmuratova and O. G. Nemtsova, Dokl. Akad. Nauk RUz, No. 12, 43 (1988).
10. F. Stierpe and L. Barbiere, Biochem. J., **240**, 659 (1986).
11. W. K. Roberts and C. P. Selitrennikoff, Biochim. Biophys. Acta, **880**, 161 (1986).
12. H. Kleinkauf and H. von Döhren, Eur. J. Biochem., **236**, 335 (1996).
13. C. Boather, Pigments of Cotton Seed, Interscience, New York (1948).
14. Z. S. Khashimova, Yu. S. Mangutova, M. É. Suslo, D. M. Beknazarova, and V. B. Leont'ev, Khim. Prir. Soedin., 83 (1994).
15. U. K. Laemmli, Nature (London), **227**, 680 (1970).
16. O. H. Lowry, N. J. Rosebrough, et al., J. Biol. Chem., **193**, 265 (1951).