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ACTION OF COTTONPLANT GLYCOPROTEINS ON THE PROLIFERATIVE ACTIVITY OF CELLS

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A mitogenic action of the total (LLPs) and lactose-specific (LLPs-1) lectin-like proteins on the proliferation of lymphocytes and of myeloma cells in culture has been found. A dose-dependent influence of the LLPs and extensin-like proteins (ELPs) on the inclusion of ^{3}H-proteins in the myeloma cells, including an antimitotic effect of the ELPs with an increase in the protein dose, has been shown; the ELPs exert an analogous action on K-562 tumor cells of human erythroblasts. The nature of the biological action of the LLPs and ELPs is discussed in connection with features of the structure of the carbohydrate fragments of the composition of the glycoproteins.

Among the functional proteins of plants, the attention of researchers is being attracted to the lectin-like and extensin-like proteins, the majority of which are glycoproteins [1-3]. It is known that glycoproteins of lectin nature cause the specific agglutination of cancer cells or arrest their development, suppressing protein synthesis [4].

Agglutination under the action of lectins is undergone by various types of blood cells — lymphocytes, leukocytes, erythrocytes, splenocytes [2, 5]; the participation of similar proteins in the protective strategy of plants has been established [6]. The polyfunctionality of these proteins is due to the diversity of the structure of the oligosaccharide fragments, which are also responsible for their biological activity.

We have investigated the influence of LLPs and ELPs on the level of synthesis of DNA, which, as is known, reflects the proliferative activity of cells.

We have previously isolated and characterized cottonplant glycoproteins, including lectin-like and extensin-like proteins [7].

Biological activity was studied on various types of cells, the level of proliferation of the cells being evaluated from the inclusion of ^{3}H-thymidine. It was established that the total LLPs exerted a stimulating influence on the proliferative activity of lymphocytes (more than 3-fold), while on combined action in the presence of Con A this effect fell by one half. It is obvious that there is mutual saturation of the mitogen-activating centers of the glycoprotein and Con A. It must be mentioned, however, that the mitogenic action of cottonplant lectins was shown at a considerably higher dose (100 μg) than for known mitogens — Con A (10 μg) and PHA (10 μg) (Table 1)

In another system for testing the biological action of cottonplant proteins we chose transplantable cell strains of XAg 8.653 myeloma and of K-562 human erythroblasts. The results of testing are given in Table 2.

Proteins from seeds, shoots, and a suspension culture of the cotton plant were added to the culture medium in doses of from 10 to 200 μg per 10^5 cells in each well. Both the total LLPs and also the lactose-specific fraction and the ELPs stimulated the proliferation of the myeloma cells, and this action had a dose-dependent nature. The appreciable stimulation of the inclusion of ^{3}H-thymidine at low doses (10-50 μg) fell when the doses were increased. The ELPs-c exhibited an effect of a different type on the myeloma cells — they showed no stimulating effect; i.e., at a dose of 10 μg the inclusion of ^{3}H-thymidine remained at the level of the control, and it fell sharply at 50 μg (about 8%). A similar antimitotic effect was shown

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TABLE 1. Influence of the Lectin-Like Proteins of the Cotton Plant on the Proliferation of Lymphocytes

Samples (doses)	Percent inclusion of ^{3}H-thymidine
Control	100.0
Con A (10 μg)	450.0
PHA (10 μg)	550.0
LLPs (100 μg)	361.0
LLPs (50 μg) + Con A (10 μg)	173.2

TABLE 2. Action of Cottonplant Lectin- and Extensin-Like Proteins on the Proliferation of Myeloma and K-562 Cells

Sample	Percent inclusion of ^{3}H-thymidine at the following doses of protein, μg			
	10	50	100	200
Control	100.0			
Con A	129.0	–	–	–
ELPs-c	108.8	8.7	–	–
ELPs-s	170.8	89.5	14.4	–
LLPs	192.3	145.5	–	121.6
LLPs-1	135.6	120.6	–	102.3
ELPs-s*	50.5	45.6	19.1	–

*ELPs-s were added to a K-562 cell culture kindly provided by N. N. Kuznetsova.

in the case of the ELPs-s, but in a higher dose (100 μg). The action of these proteins was also checked on the K-562 cell line. It can be seen from Table 2 that the ELPs-s exerted an inhibiting action on erythroblast cells, this being most pronounced at a dose of 100 μg. On the other hand, these cells proved to be insensitive to the action of the lectin-like proteins (results not given).

Thus, both lymphocytes and tumor cells in culture showed sensitivity in various degrees to the action of cottonplant proteins. The difference in the natures of the action of LLPs and ELPs that we have detected are apparently connected with the type of cells and with features of the structures of the proteins studied. Thus, electrophoretic analysis of the lectin-like proteins in 0.1% SDS-PAAG with the use of Con A peroxidase followed by staining the gel with diaminobenzidine showed the presence of glycoproteins as components of the LLPs [8]. In addition, we determined the carbohydrate contents of the LLPs, LLPs-1, and ELPs: LLPs — 11.6%; LLPs-1 — 18.0%; the ratio of protein to total carbohydrates for the ELPs was approximately 1:4.

Earlier, by cross-immunochemical analysis using monoclonal antibodies, we established the overlapping of clone 1C6 with the LLPs and LLPs-1 and also with the reaction of known lectins (Con A and RCA-120). This permitted the assumption that structures with β-*D*-galactosyl terminals and also with carbohydrate chains of the oligomannoside and complex types were present in the antigenic determinants of the proteins [7]. On the other hand, proteins belonging to the extensin group have a completely different type of structure of the carbohydrate fragments — arabinogalactan chains of extended form [9].

The stimulating effects on the proliferation of cells of low doses of both groups of proteins and, on the other hand, the antimitotic action of the ELPs at high doses obviously reflect the nature of the interaction of these proteins with the surface of cells (lymphocytes, myeloma, K-562). The latter is due to peculiar features and the specific nature of the structure of the carbohydrate fragments of the glycoprotein components of the LLPs and ELPs and also of the membrane receptors of the target cells, the interaction of which, together with other factors, also ensures the mechanism of the mediation of the proliferative response of the cells.

EXPERIMENTAL

The lectin-like proteins (LLPs) of cotton seeds were isolated by extraction with salt solutions from seed flour that had been treated for defatting and the elimination of pigments, followed by stepwise salting-out with ammonium sulfate in the

interval of 30-80% saturation. The lactose-specific proteins (LLPs-1) were obtained by the affinity chromatography of the total LLPs on Sepharose 4B with desorption by 0.1 M *D*-lactose [7].

The extensin-like proteins (ELPs) were isolated from a suspension culture (ELPs-c) and from two-day shoots (ELPs-s) of the cotton plant by extraction with 0.2 M $CaCl_2$ using a modification of Lamport's method [3]; the further purification of the protein was achieved on a column of CM-cellulose.

The amounts of total sugars in the proteins were determined spectrophotometrically and with the anthrone–sulfuric acid reagent. For this purpose, 3 ml of the anthrone reagent (a 0.18% solution of anthrone in concentrated sulfuric acid of OCCh [ultrapure] grade) was added to 1 ml of protein solution; the mixture was incubated for 10 min in the boiling water bath, and, after cooling, the absorption at 620 nm was measured. *D*-Glucose was used as calibration standard.

Protein contents were determined by Lowry's method [10]

To determine proliferative activity we used lymphocytes from peripheral human blood and also transplantable cell strains of XAg8.653 myeloma and K-562 human erythroblasts.

The lymphocytes were isolated by a standard procedure, using a Ficoll–Hypak gradient. The lymphocytes were incubated in 24-well plates with 10^5 cells per well in a thermostat with 5% CO_2 for two days in RPMI-1640 medium containing 20% of inactivated embryonic calf serum and antibiotics.

The myeloma and K-562 cells were sown in 24-well plates at a density of 100 thousand cells per well in RPMI-1640 medium with 10% of serum and antibiotics. The proteins were dissolved in the same growth medium without antibiotics. For the growth of the cell cultures we used Flow Labs reagents and plastic vessels.

Cells were incubated with the preparations for 24 h in an atmosphere of absolutely humid air with 5% of CO_2. After 4-6 h and before the end of cultivation, 10 μCi of Amersham ^{3}H-thymidine in a volume of 10 ml was added to each well.

Control cells were incubated in the same growth medium without the preparations. All the experiments, including the controls, were set up in three parallel wells.

To estimate the levels of inclusion of radioactivity, 300 μl of resuspended mixture was taken from each cell 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 (Beckman, SL-230).

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