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A STUDY OF THE MECHANISM OF THE ACTION OF EXTENSIN-LIKE PROTEINS OF THE COTTON PLANT IN A CELL CULTURE

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The mechanism of the action of extensin-like proteins (ELPs) isolated from the cotton plant on a cell line of murine melanoma (CML) has been studied from the inclusion of ^{3}H-thymidine and by counting live cells. The dose- and phase-dependent inhibitory nature of the action of ELPs on MLC cells has been established.

The extensin-like glycoproteins (ELPs) of plants participate in the formation of the primary cell wall, and their effect appears most clearly under stress conditions. The actions of pathogens, the temperature, and ethylene cause an increase in the level of these proteins [1—5]; some of them possess an agglutinating activity, like the lectins. Cytotoxic properties of the lectins both at the membrane and at the intracellular levels [6] and their participation in the aggregation and adhesion of normal and tumor cells [7] are known. However, the mechanism of the action of the extensin-like proteins has not been studied.

We have previously shown an antiproliferative activity of cottonplant ELPs in relation to various types of plant and animal cells: a suspension culture of cells of the cotton plant, a culture of cells of murine myeloma Ag 8.653, and K-562 cells [8, 9].

The task of the present work was to investigate the mechanism of the action of ELPs on the biological activity of a stable line of cells of murine myeloma (CML).**

We used ELPs that we had isolated from a callus of a cotton plant by extraction with 0.2 M EDTA and characterized electrophoretically [8]. The proteins possessed no hemagglutinating activity. The ratio of protein and total sugars was 1:3.

To study the mechanism of the action of ELPs and also the capacity of these proteins for causing the aggregation of cells in a culture, we took a highly proliferating culture of CML cells. The cells were sown at the rate of 100 thousand per flask containing 2 ml of nutrient medium RPMI-1640 with 10% of serum and antibiotics. The EPLs were added in doses of 10, 50, and 100 μg/ml and the action of the preparations was determined after a day from the inclusion of ^{3}H-thymidine in the cells and by counting live cells. Cells grown in the absence of a preparation were used as controls. The results obtained are given in Table 1.

As can be seen from Table 1, the action of the ELPs on CML cells depends on the dose. At a dose of 100 μg/ml the inclusion of the label was suppressed by 51% (CE_{50}); at 50 μg/ml, by 27%; and at 10μg/ml, by 7%. An analogous effect was observed by counting live cells. Thus, at a dose of 100 μg/ml cell growth was suppressed by 48%; at 50 μg/ml, by 23%; and at 10 μg/ml, by 4%, it being shown that the cells were not depressed and not disrupted but could be stained by Trypan Blue — i.e., they were dead. In this way it was shown that the action of the ELPs on CML cells is dose-dependent and is expressed as a suppression of the proliferative activity of the cells.

*Deceased.

**The stable line of murine melanoma cells was obtained in the Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, in 1996 from a transplantable strain of murine melanoma V-16 by H. H. Kuznetsova, and we have called it CML.

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TABLE 1. Action of ELPs on a Culture of CML Cells

Dose of ELPs, μg/ml	Suppression of the inclusion of ^{3}H-thymidine, %	Suppression of cell growth*,%
100	51	48
50	27	23
10	7	4

*The suppression of cell growth was calculated from the percentage of live cells.

TABLE 2. Suppression of the Growth of CML cells in the Logarithmic and the Stationary Phases*

Sample	Dose, μg/ml, CE_{50}	Suppression of Cell Growth, %	
		log. phase	stat. phase
ELPs	100	49.0	8.0
Sarcolysine	15	44.0	2.0
Vincristine	1	54.0	12.0
Sarcolysine + vincristine	15+1	80.0	-
ELPs + sarcolysine	100+15	62.0	-
ELPs + vincristine	100+1	85.0	-

*The suppression of cell growth was calculated from the percentage of live cells.

We have also established that cell aggregation takes place under the action of ELPs. It is possible that aggregation is one of the stages in the suppression of cell proliferation and is connected with a protective reaction of the cells to the aggression of the ELPs.

For a tool in a more detailed study of a possible mechanism of the action of ELPs in a culture of CML cells, we used compounds with a known action mechanism. One of them is sarcolysine — *D,L*-α-amino-β-(*para*-{bis[chloroethyl]amino} phenyl) propionic acid hydrochloride — which alkylates DNA, and another is vincristine — an alkaloid of the indole series isolated from *Vinca rosea* L., which blocks cell mitosis at the metaphase stage. We also showed that 50% suppression of cell growth (CE_{50}) requires 15 μg/ml of sarcolysine or 1 μg/ml of vincristine. The action of thee compounds was studied in a partially synchronized culture of CML cells (the time of cell generation was 14—16 h). The ELPs and the above-mentioned drugs were added to the cell culture separately or together in 50% doses in the logarithmic and the stationary phases of the growth of the cell culture. The results obtained are given in Table 2.

In the logarithmic phase of the growth of the cells, under the combined action of sarcolysine and vincristine we observed 80% suppression of the proliferation of cell growth, which indicates a cytotoxic action of the drug covering two phases of cell division — the S period (replication of DNA) and mitosis. Under the combined action of the ELPs and sarcolysine, taken in 50% doses (100 μg and 15 μg) we observed 62% suppression of cell growth; i.e., the ELPs, just like sarcolysine, act mainly on the S period of cell division. In the case of ELPs with vincristine the effect of suppression was analogous to the action of sarcolysine with vincristine on cell proliferation. It must be assumed that the ELPs and vincristine suppress cell growth in two phases — the S period and mitosis.

In the stationary phase (stagnation) of cell growth the ELPs, sarcolysine, and vincristine were practically inactive — 8, 2, and 12%, respectively.

Thus, the results that we have obtained show that the action of ELPs on cells is dose-dependent, and the observations on the combined action of ELPs with sarcolysine and with vincristine permit the assumption that the extensin-like proteins isolated from the cotton plant act mainly in the S-period of cell division when transcriptionally active chromatin is most vulnerable to external actions.

EXPERIMENTAL

The extensin-like proteins were isolated by us from a callus of the cotton plant by extraction with 0.2 M EDTA and were characterized electrophoretically as described previously [9].

Total sugars were determined by the anthrone/sulfuric acid method [9].

The hemagglutinating activity of the ELPs was determined with a 2% suspension of human or murine blood without the use of trypsin [10].

The transplantable line of CML cells was obtained from a tumor strain of murine melanoma B-16 of the C57BL line. The tumor was excised under sterile conditions and was homogenized in RPMI-1640 medium with an excess of antibiotics (penicillin, gentamicin, nystatin). The cell suspension was washed twice with nutrient medium and was centrifuged at 1.2 thousand rpm for 10 min in a MLWK-23. The cell deposit was resuspended in RPMI-1640 medium with 15% of embryonic serum, glutamine, and antibiotics (complete medium) and was sown in Carrel flasks in a concentration of 200 thousand cells per 1 ml. A stable cell line was obtained in 6 months.

Treatment of the Cells. The cells were washed off the glass with a 1 mM solution of Versene (EDTA) and were sown at 100 thousand cells/ml in a volume of 2 ml of complete medium in penicillin flasks at 37°C. After 24 h, the ELPs, sarcolysine, and vincristine were added separately and in combination in 1 ml of complete medium. The compounds were left in contact with the cells for 24 h, after which ^{3}H-thymidine (30 μCi per flask) was added or the live cells were counted.

After incubation with the label, the medium was poured off, 1 ml of 1 mM EDTA was added over 3—5 min, the cells were suspended and transferred to GFC filters, and these were fixed, washed with 5% TCA, dried, and counted in a scintillate (PPO—POPOP—toluene) in a liquid scintillation counter.

Cells taken up with 1 mM EDTA were stained with 0.4% Trypan Blue and an aliquot was transferred to a Goryaev chamber, where the live cells were counted under a microscope.

All the experiments, including the controls, were carried out in triplicate.

Protein contents were determined by the Lowry method [11].

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