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EFFECT OF EXTENSIN-LIKE COTTON PROTEINS ON EUKARYOTIC CELL GENE

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The effect of extensin-like cotton proteins (ELP) on animal cell gene (KML cell culture) was studied. Cells perish (50%) at protein dose 100 μg/mL (cytotoxic effect). DNA fragmentation is not observed. Cell death from ELP is hypothesized to occur via necrosis resulting from destruction of intracellular biochemical processes.

Key words: extensin-like proteins, cotton, cell death, DNA, necrosis, apoptosis.

Cell death and proliferation play important roles in normal development of an organism [1, 2]. Loss of control of cell death during growth and development of tissues and organisms can induce various pathological states: generation of malignant neoplasms, impaired immune response, etc. [3]. It was found that cells perish via necrosis or apoptosis. The process is induced by various factors, e.g., toxic substances, irradiation, temperature, apoptogens, etc. Therefore, the nature of the primary signal varies [4-6]. Several lectins exhibit cytotoxic properties at the membrane and within cells [7]. The effect of carbohydrate-containing proteins, in particular, lectins, is due to specific binding of oligosaccharide antennae to carbohydrate components of cellular receptors, which initiates a cascade of intracellular processes that reach the nucleus itself [8]. Extensin-like proteins (ELP) are interesting because they are a glycoproteide of the plant cell wall. ELP participate in cell-wall construction as structural proteins and undergo changes. Thus, their content increases sharply when cells are stressed, indicating that they have a protective function [9-11]. However, the mechanism of action of these proteins is practically unstudied.

We investigated the effect of cotton ELP on cell genetic material. ELP were isolated from 2-day sprouts of *G. hirsutum* L. and were characterized by electrophoresis. The carbohydrate content was ~20%. Traces of phenolic compounds were not detected. The proteins do not exhibit hemagglutinating activity [12, 13].

We found previously that cotton ELP are cytotoxic according to ^{3}H-thymidine incorporation to various types of plant and animal cells: a suspended culture of *G. hirsutum* L. cells and cultures of Ag 8.653 murine myeloma and K-562 human erythroblastosis cells [14].

The antiproliferative activity of ELP on the rapidly proliferating KML cell line obtained by us from B-16 murine melanoma was demonstrated using ^{3}H-thymidine incorporation [15]. A dose-dependent inhibition of DNA synthesis was noted. The inhibition of DNA synthesis in KML cell culture by ELP was:

Sample	ELP dose, μg/mL	Suppression of ^{3}H-thymidine incorporation, %
1	100	51
2	10	7

The possible mechanism of ELP action was determined using preparations of known activity (sarcolysin, which aklylates DNA and acts on the S-period of cell division, and vincristine, which suppresses cell division in mitosis) in logarithmic and static phases of the cell cycle. Experiments on the separate and combined action of ELP and known preparations revealed that the point of ELP involvement is the S-period of the cell cycle when chromatin is transcriptionally active and most vulnerable

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to various factors [16].

The nature of ELP suppression of DNA synthesis was established by distributing KML cells (80,000) in flasks in RPMI-1640 nutrient medium (2 mL) and adding protein in doses of 10 and 100 μg/mL. Cells were contacted with the ELP for 24 h. Live cells were counted by decanting the nutrient medium (fraction 1) and carefully pipeting versene to remove cells from the glass. A part of the cells (50 μL) was selected for counting live cells using trypan blue dye. Averages of 52 and 95% of the cells remained viable at protein doses of 100 and 10 μg/mL, respectively.

Thus, the antiproliferative activity of ELP that is estimated from ^{3}H-thymidine incorporation is accompanied by cell death. The morphology of KML cells does not differ visibly from that of controls. Cytological analysis of KML cells showed cell aggregation at a protein dose 100 μg/mL that was not previously described. This may be due to a protective reaction of the cells against ELP.

Cells are known to swell during necrosis. The intracellular organelles expand (owing to malfunction of ion channels) and burst. The cells have perished because they are dyed by trypan blue. During apoptosis, chromatin becomes supercondensed and DNA starts to fragment. The cell is still living at this stage (cells are not dyed by trypan blue) and then is phagocytized by macrophages or neighboring cells [1, 2, 4, 17, 18].

DNA becomes fragmented during apoptosis. Therefore, all fractions (1, 2, and 3) were collected. The DNA content was determined using diphenylamine (DPA) as dye. Precipitated DNA was characterized by electrophoresis in agarose gel (0.5%) [19].

The results show that all precipitated fractions (1, 2, and 3) do not contain fragmented DNA. The characteristic "ladder" produced by DNA fragmentation is not observed in the electrophoregrams.

Thus, cells aggregate, 50% of ^{3}H-thymidine incorporates, the same fraction of cells is colored by trypan blue, and the electrophoregram shows one diffuse band similar to the control at the given protein dose (100 μg/mL). Cell death from ELP is proposed to occur via necrosis due to disruption of intracellular biochemical processes.

EXPERIMENTAL

ELP were isolated from 2-day cotton sprouts. They were characterized by us previously by electrophoresis [13]. The contents of carbohydrate and phenolic compounds were determined as before [12].

Cells were prepared, the radioactivity level was measured, or live cells were counted as described in the literature [16].

Isolation of DNA. Cells were centrifuged for 5 min in an Eppendorf (Beckman) centrifuge. The supernatant liquid was decanted (fraction 2). The precipitate was suspended in tris-HCl buffer at pH 6.8. Sodium dodecylsulfate (Na-DDS) was added. The mixture was centrifuged. The supernatant liquid was decanted (fraction 3). The precipitate was suspended in tris-HCl buffer containing NaCl (0.14 M). An equal volume of phenol (1 mL) was added. The mixture was shaken. The upper aqueous layer was carefully decanted after centrifugation. Isoamyl alcohol was added. The mixture was shaken and centrifuged. The aqueous layer was decanted, treated with ten times the volume of cold alcohol, and centrifuged. The precipitate of DNA was dissolved in tris-HCl buffer and used for electrophorsis analysis. Electrophoresis was performed in agarose gel (0.5%) [19].

Supernatant liquid (1 mL, fractions 1, 2, and 3) was treated with twice the volume of DPA solution. The mixtures were refluxed for 10 min on a boiling-water bath and then cooled. The DNA contents were determined spectrophotometrically using an orange light filter. Commercial DNA preparation was used as a control.

DPA solution was prepared as follows. DPA (1 g) was dissolved in glacial acetic acid (100 mL) containing conc. H_2SO_4 (2.75 mL). Freshly prepared solution was used.

Experiments including controls were performed in triplicate.

The protein content was determined by the Lowry method [20].

REFERENCES

1. J. F. R. Kerr, A. H. Wyllie, and A. R. Currie, *Br. J. Cancer*, **26**, 239 (1972).
2. S. R. Umanskii, *Mol. Biol.*, **30**, 487 (1996).
3. C. B. Thompson, *Science*, **267**, 1456 (1995).

4. M. P. Kutsyi, E. A. Kuznetsova, and A. I. Gaziev, *Biokhimiya*, **64**, 149 (1999).
5. M. Enari, H. Hug, and S. Nagata, *Nature (London)*, **375**, 78 (1995).
6. G. A. Romanov, A. P. Kuzmenko, V. S. Dashkevich, R. I. Salganik, and B. F. Vanyuchin, *FEBS Lett.*, **165**, 35 (1984).
7. V. M. Lakhtin, *Biotekhnologiya*, **5**, No. 6, 676 (1989).
8. H. R. MacDonald and M. Nabholz, *Annu. Rev. Cell Biol.*, **2**, 231 (1986).
9. J. M. Daly, *Annu. Rev. Phytopathol.*, **2**, 273 (1984).
10. D. A. Stuart and J. E. Varner, *Plant Physiol.*, **66**, 787 (1980).
11. J. R. Ecker and R. W. Davis, *Proc. Natl. Acad. Sci. USA*, **84**, 5202 (1987).
12. Z. S. Khashimova, N. N. Kuznetsova, O. Kh. Saitmuratova, and A. A. Sadykov, *Khim. Prir. Soedin.*, 331 (2000).
13. Z. S. Khashimova, Yu. S. Mangutova, M. E. Suslo, and V. B. Leontʹev, *Fiziol. Rast.*, **47**, 216 (2000).
14. Z. S. Khashimova, Yu. S. Mangutova, M. E. Suslo, D. M. Beknazarova, and V. B. Leontʹev, *Khim. Prir. Soedin.*, 83 (1994).
15. N. N. Kuznetsova, Z. I. Mardanova, Z. S. Khashimova, V. B. Leontʹev, and A. A. Sadikov, *Cultivated Murine Melanoma Cells KML* [in Russian], Uzb. Pat. (2001).
16. Z. S. Khashimova, N. N. Kuznetsova, Z. I. Mardanova, and V. B. Leontʹev, *Khim. Prir. Soedin.*, 372 (1999).
17. A. H. Wyllie, *Nature (London)*, **284**, 555 (1980).
18. D. G. Brown, X.-M. Sun, and G. M. Cohen, *J. Biol. Chem.*, **268**, 3037 (1993).
19. T. Maniatis, E. F. Fritsch, and J. Sambrook, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1982).
20. O. H. Lowry and F. J. Rosenbrough, *J. Biol. Chem.*, **193**, 265 (1961).