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BIOLOGICALLY ACTIVE PROTEINS OF THE WORM *Eisenia foetida*

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Seven protein-peptide fractions have been isolated from the biomass of the worm Eisenia foetida. *Their influence on the synthesis of RNA, DNA, and protein in animal and plant cells has been investigated.*

The biomass of earthworms is attracting the attention of many researchers as a potential source of physiologically active compounds. Hypolipidemic, antidiabetic, and antithrombolytic properties have been found for the biomasses of earthworms of the genera *Lumbricus, Eisenia, Allolobophora, Dendrobaena, etc.* [1]. There are also reports on the presence of antitumoral and immunostimulating factors [2]. However, these compounds have not been identified and the mechanism of their action has not been studied.

We have isolated proteins and peptides from *Eisenia foetida* and have investigated their influence on the synthesis of DNA, RNA, and protein. For this purpose, an aqueous extract of a homogenate of the worms was separated into a fraction containing proteins of high molecular mass and a fraction containing components of low molecular mass.

The fractions obtained were tested for cytotoxic activity in vitro on tumor cells of Ehrlich's ascitic carcinoma. Both fractions inhibited the inclusion of [^{3}H]thymidine in DNA (by 46.2 and 43.4%, respectively) and of [^{3}H]uridine in RNA (by 65.5 and 52.3%, respectively). The low-molecular-mass protein-peptide fraction was separated by hydrophobic chromatography on the sorbent Lichroprep in an isopropanol gradient (Fig. 1). Seven hydrophobic fractions were obtained, and these were also tested for cytotoxic activity (Table 1).

As follows from the results obtained, together with fractions inhibiting the synthesis of RNA and DNA, there were fractions activating this process. Contrary to expectations, the inhibiting effect of the total fraction proved to be higher than that of the individual components. A possible explanation of this phenomenon may be a synergistic interaction. It is too early to draw definitive conclusions since it has not been possible to determine the activity of the 7th fraction, which is insoluble in water or in the culture liquid because of its high hydrophobicity.

On the basis of the fairly close organization of animal and plant chromatin, we assumed that the protein-peptide fractions from *Eisenia foetida* might prove to be promising regulators of processes in the plant cell. We have investigated the influence of these fractions on the synthesis of protein in isolated cottonplant chromatin (Table 2).

It was found that the plant chromatin reacted in almost the same way as the tumor cell chromatin to the presence of the protein-peptide fractions under investigation in the reaction mixture, and the activity of the 7th fraction far exceeded that of a well known inhibitor of RNA polymerase — the antibiotic rifampicin. It is not excluded that this fraction contained an antibiotic of protein nature.

Thus, with respect to their functional activities the fractions obtained are a promising source of protein-peptide bioregulators and molecular-genetic markers in the creation of transgenic varieties of cotton plant with given properties.

EXPERIMENTAL

Isolation of the Fraction of Proteins and Peptides. The worm biomass (100 g) was washed with water, kept in 1 liter of 0.05 M citrate buffer, pH 6.00 at 10°C for 3 h, washed with water, and homogenized, first in a tissue grinder, and

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TABLE 1. Influence of the Protein-Peptide Fractions from *Eisenia foetida* on the Synthesis of DNA and RNA in Tumor Cells of Ehrlich's Ascitic Carcinoma (dose 60 μg/ml)

Fraction No.	Inclusion of [^{3}H]-thymidine, %	Inclusion of [^{3}H]-uridine, %
	DNA	RNA
Control	100	100
1	145,5	104
2	20,5	7
3	20	0,8
4	26	3
5	103,5	105
6	5.5	10
7	Insoluble	Insoluble
Total	43,4	52,3

TABLE 2. Influence of the Protein-Peptide Fractions of *Eisenia foetida* on the Synthesis of Protein in Isolated Cottonplant Chromatin (dose 60 μg/ml)

Fraction No.	Inclusion of [^{35}S]-methionine, %	Fraction No.	Inclusion of [^{35}S]-methionine, %
Control	100	5	116
1	133	6	92
2	103	7	7
3	100	Total	15
4	89	Rifampicin	32

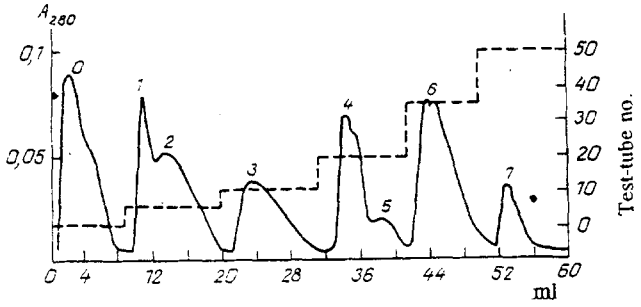

Fig. 1. Hydrophobic chromatography of the total protein-peptide fraction. Column (1.0 × 10 cm) of Lichroprep C-8; buffer 0.1 TFAA, with a stepwise (5, 10, 20, 35, and 50%) gradient of isopropanol at a rate of flow of 10 ml/h.

then in a Potter homogenizer. Water was added to the homogenizate in a ratio of 2:1, and the mixture was stirred and was filtered through Kapron [polycaprolactam] fabric. Acetone was added to the filtrate in a 1:1 ratio, and it was kept at 4°C for 10 h. The resulting precipitate was removed by centrifugation at 6000 *g* for 30 min. After the elimination of the acetone, the supernatant was lyophilized. This gave 5 g of total protein-peptide fraction.

Hydrophobic Chromatography. The total fraction was separated by hydrophobic chromatography on the sorbent Lichroprep C8 in a 10-ml column with a stepwise concentration gradient of isopropanol. Rate of flow 20 ml/h; absorption at 280 nm.

Cytoxic activity was determined by two methods.

1. From the Inhibition of the Synthesis of DNA using (^{3}H)thymidine. Each well of a planchet for immunological reactions was charged with 90 μl of a cell suspension (8×10^5 cells/ml), and 10 μl of one of the solutions under test was added. [^{3}H]Thymidine was added to the cell suspension in an amount of 1.5 μCi/ml. Incubation was carried out at 37°C for 2 h. The samples were deposited on filters, dried in the air, and washed successively with cold TCAA solution (4×5 ml) and 96% ethanol (2×5 ml per filter). After drying, radioactivity was recorded in a toluene scintillator on a Rackbeta counter (LKB, Sweden).

2. From the Inhibition of the Synthesis of RNA using [^{3}H]Uridine. Each well of a planchet was charged with 90 μl of a cell suspension (8×10^5 cells/ml) with 1 μCi of [^{3}H]uridine per ml, and 10 ml of the solution under test was added. Incubation was carried out at 37°C for 2 h. The subsequent operations were as described in the preceding method. As a model in both methods we used tumor cells of Ehrlich's ascitic carcinoma taken from mice 6-8 days after the inoculation of the tumor.

The protein-synthesizing activity of isolated cottonplant chromatin was determined by the method of Wielgat and Kleczkowski [3]. As the radioactive marker we used (^{35}S)methionine produced by the Radiopreparate AP (Tashkent) with a molar activity of 8 PBq/mole.

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