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ISOLATION AND PRIMARY STRUCTURE OF CYTOTOXIN FROM THE CALIFORNIA RED WORM *Eisenia foitida*

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Earthworm biomass has been used since antiquity as medicine for various diseases. Antitumor, immunostimulating, hypolipidemic, antidiabetic, and antithrombolytic properties of biomass from *Lumbricus*, *Eisenia*, *Allolobophora*, *Dendrobaena*, and other earthworms have been reported [1, 2]. However, the mechanism of action of the components has not yet been identified and studied. It was previously reported that reverse-phase chromatography on Lichroprep C8 sorbent isolated seven fractions designated R-1-1 through R-1-7 from the California red worm *Eisenia foitida*. The cytotoxic activity of these fractions was investigated using *in vitro* inhibition of ^{3}H-thymidine incorporation into DNA and ^{3}H-uridine incorporation into RNA of ascitic Erlich carcinoma tumor cells [3].

Preparative quantities of the peptide fractions were isolated using reverse-phase chromatography on silochrom 80-C_{18} sorbent that we synthesized [4]. Fraction R-1-2, which was eluted with 10% isopropanol, was the most active and reduced incorporation of ^{3}H-thymidine into DNA by 20.5% and of ^{3}H-uridine into RNA by 7% (50 μg/ml). Fraction R-1-2 was further separated by gel filtration on a column with TSK HW 40f sorbent equilibrated with ammonium acetate (0.05 M) buffer (pH 5.4). This gave six fractions, of which fraction R-1-2-5 (50 μg/ml) inhibited incorporation of ^{3}H-thymidine into DNA by 41% and ^{3}H-uridine into RNA by 20%. Further purification of fraction R-1-2-5 by HPLC on a Nucliosil C_{18} column using a linear gradient of CH_3CN from 1-40% in 0.1% F_3CCOOH produced 20 fractions.

Investigation of the resulting fractions found cytotoxic activity in R-1-2-5-3 and R-1-2-5-12. Further study showed that these were homogeneous peptides (5 μg/ml) that inhibited incorporation of ^{3}H-thymidine into DNA (by 100 and 97%, respectively) and ^{3}H-uridine into RNA (by 83 and 11%, respectively). The N-terminus of R-1-2-5-3 is isoleucine; of R-1-2-5-12, alanine [5]. The molecular masses of the peptides were found on a previously calibrated TSK 2000 SW (8 × 300 mm) column. They were 1000 Da (R-1-2-5-3) and 1200 Da (R-1-2-5-12). Analysis of PTC derivatives of the aminoacids produced by total acid hydrolysis of the isolated peptides using HPLC on a 100/7/4 SuperPac-3 C_{18} column detected the following aminoacids in the peptides:

R-3-3: Gly 1.0 (1), Thr 0.7 (1), Ala 1.1 (1), Agr 1.6 (2), Val 0.7 (1), Ile 0.7 (1), Lys 0.8 (1). Total 8.

R-3-12: Asp 0.7 (1), Ala 1.0 (1), Arg 1.2 (1), Val 0.8 (1), BKM-Cys 3.8 (4), Phe 1.2 (1), Lys 0.9 (1). Total 10.

The aminoacid sequences of the peptides were determined by the Edman and dansylation methods [6, 7]. The sequences of five aminoacids in R-3-3 and three aminoacids in reduced and carboxymethylated R-3-12 were determined using the Edman method for direct identification of PTH-derivatives of aminoacids on a DuPont chromatograph on a 100/7/4 SuperPac-3 C18 column [8] and also in combination with dansylation and identification of the N-terminal aminoacids of cleaved peptides on silica-gel plates. The analysis found the following aminoacid sequences:

R-3-3: Ile-Val-Thr-Gly-Arg-...

R-3-12: Ala-Val-Asp-...

The C-terminal aminoacid sequence was found using hydrolysis by carboxypeptidase Y with an enzyme:substrate ratio of 1:100 [9]. Aliquots were taken after 20, 40 and 60 min. Phenylisothiocyanate was added. The PTC-derivatives of the separated aminoacids were analyzed by HPLC. The data show that the C-terminal aminoacid sequence of R-3-3 is ...Lys-Arg-Ala-COOH. Results of the N- and C-terminus analysis enabled the structure of R-1-2-5-3 to be reconstructed:

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1 2 3 4 5 6 7 8

H_2N - ILE - VAL - THR - GLY - ARG - LYS - ALG - ALA - COOH

→ → → → → ← ← ←

The C-terminal aminoacid sequence of R-1-2-5-12 could not be determined.

Thus, two cytotoxic peptides that inhibit the incorporation of ^{3}H-thymidine into DNA and ^{3}H-uridine into RNA are isolated for the first time from an extract of *Eisenia foitida*. The study of the primary structure of R-1-2-5-3 using the Edman method and the determination of the C-terminal aminoacid sequence using carboxypeptidase Y enabled the total primary structure to be established. The primary structure of R-1-2-5-12 is partially determined.

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