BIOLOGICALLY ACTIVE GLUTAMYLPEPTIDE FOUND IN RABBIT BONE MARROW

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UDC 612.419.015.2

A biologically active glutamylpeptide with unusual rotation of the polarization plane, opposite to that observed for natural proteins, has been found in rabbit bone marrow and the technique of its isolation in a chromatographically pure form is described. In a concentration of 10^{-5} M the isolated peptide reduces the survival rate of Chinese hamster fibroblast-like cells in culture by 70% and in certain physicochemical properties it resembles the glutamylpeptide with cytogenetic activity and capable of potentiating the mutagenic action of ionizing radiation, previously isolated from plants.

KEY WORDS: bone marrow tissue; biologically active substances; peptides; mutagens.

In the course of a search for natural mutagens and modifiers of radiation mutagenesis in dividing plant cells and the investigation of their properties the writers found a substance capable of inducing the formation of a certain type of chromosomal injury and of potentiating the induction of chromatid and chromosomal aberrations by radiation in plant cells [6, 7] and also of causing reproductive death of Chinese hamster cells in culture [3]. The compound isolated in the chromatographically pure form was found to be a low-molecular-weight glutamylpeptide, with an unusual rotation of the polarization plane, in the opposite direction to that observed for natural proteins.

Considering the unusual biological and optical properties of the substance isolated it was decided to study its distribution in the dividing cells of other organisms. Bone marrow cells, deciding the fate of the whole animal after irradiation with corresponding doses of ionizing radiation, were chosen as the test object.

EXPERIMENTAL METHOD

Femurs of rabbits aged 4-5 months were frozen in liquid nitrogen, the marrow was removed and suspended in water, and the floating fat was skimmed off. The suspension was homogenized and extracted three times with ethanol in a final concentration of 75%. The extracts were pooled, dried, and dissolved in distilled water, frozen and thawed twice, and then centrifuged. The supernatant was freeze-dried and fractionated as described below. The localization of the separated substances was determined by treating part of the chromatogram with chlorine–iodide reagent for peptides [1] and also by examining it in UV light. The located substances were eluted from the chromatogram with distilled water and freeze-dried. The amino-acid composition was determined with the "Biocal" (West Germany) amino

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Laboratory of Molecular Radiobiology, Institute of Biological Physics, Academy of Sciences of the USSR, Pushchino-on-Oka. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 12, pp. 84-86, December, 1975. Original article submitted February 17, 1975.

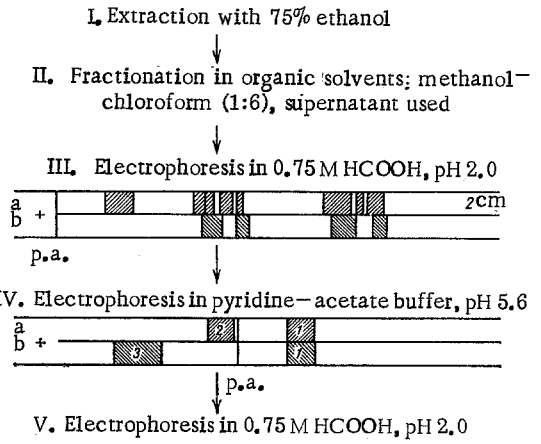

Fig. 1

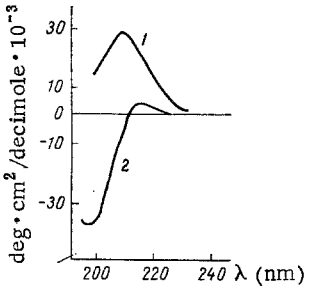

Fig. 2

Fig. 1. Scheme for fractionation of glutamylpeptide from rabbit bone marrow: I-V) stages of fractionation; a) position of luminescent fractions and fractions absorbing in UV light on chromatograms; b) location of fractions giving positive chlorine–iodide test; 1, 2, 3) Nos. of fractions; p.a.) point of application; +, –) anode and cathode, respectively; *) fraction used for further purification. [As in Russian original: The * does not appear in Fig. 1 – Consultants Bureau.]

Fig. 2. Circular dichroism of glutamylpeptides at pH 7.0: 1) glutamylpeptide isolated from rabbit bone marrow; 2) synthetic α-poly-L-glutamic acid [8]. Abscissa, specific ellipticity (in deg· cm^2/decimole · 10^{-3}); ordinate, wavelength (in nm).

acid analyzer after acid hydrolysis of the test substance. Optical activity was recorded on the I-20 spectropolarimeter (Japan). Biological activity of the isolated peptide was determined from the decrease in survival rate of transplanted Chinese hamster cells [5].

The freeze-dried extract was dissolved in methanol and centrifuged, chloroform was added to the supernatant in the ratio of 6:1, and after further centrifugation the supernatant was dried, dissolved in distilled water, and fractionated by electrophoresis on paper, initially in 0.75 M formic acid at pH 2.0 for 10-12 h under a voltage of 400 V, then in pyridine–acetate buffer, pH 5.6 (23 ml pyridine, 6 ml acetic acid, 970 ml water) for 2 h under the same voltage.

EXPERIMENTAL RESULTS AND DISCUSSION

A peptide with the same mobility as the plant glutamylpeptide was found on the chromatograms (Fig. 1). The yield of the peptide in question, when purified from pyridine by repeated electrophoresis in formic acid for 2 h, was about 2 μg/g of the original tissue.

Comparison of the physicochemical properties of the isolated peptide and the plant peptide was homogeneous in several solvent systems, and on fractionation by ascending chromatography on FN-17 (East Germany) paper in acetic acid:water (6:4), butanol:acetic acid:water: pyridine (15:3:12:10), and isoamyl alcohol:pyridine:water (7:7:6) systems it had R_f values of 0.90, 0.50, and 0.35, respectively, close to that for the plant peptide in the same systems. However, on chromatography in a system of butanol:ethanol:water (4:1:1) the test peptide had a much lower R_f value (0.2), indicating definite differences in structure from that of the plant peptide (R_f = 0.6). Investigation of the amino-acid composition showed that the peptide from the animal tissue also consisted practically entirely of glutamic acid residues. Only traces of glycine, alanine, and aspartic acid were found. Since the test peptide was eluted after fractionation of the initial extract on a column with Sephadex G-25 (2 × 100 cm, 300 ml, eluent water) in a volume of 190-240 ml, close to the elution volume of the plant peptide [4], it can be concluded that they have similar molecular weights, of roughly 500-2000 [2]. Determination of the optical activity of the isolated peptide

showed that this compound, like the plant peptide, has an unusual right direction of rotation. As Fig. 2 shows, the isolated peptide has positive rotation. Since the peptide consists chiefly of glutamic acid residues, it is presumably the D-isomer. The specific ellipticity value at the maximum does not suggest the existence of any considerable quantity of structural coiling in the isolated compound. The slight displacement of the maximum compared with the homopolymer formed by L-amino-acid residues is evidently explained by differences in the degree of polymerization of the specimen.

Comparison of the biological activity of these compounds was particularly interesting Since the plant peptide, as the authors described previously [3], can induce reproductive death and reduce the survival rate of Chinese hamster fibroblast-like cells in culture, the activity of the peptide isolated from marrow was tested on the same object. The concentration of peptide isolated from bone marrow cells, $1.5 \cdot 10^{-5}$ M (6.5 μg/ml) was comparable with the concentration of that from plants ($2.0 \cdot 10^{-5}$ M), sufficient to exhibit a definite biological effect, namely reducing the survival rate to 33% (40% for the plant peptide). The chemical supermutagen N-nitroso-N-methylurea, in a concentration of 10^{-5} M had a biological action of about the same magnitude [5]. These results indicate that the test peptide also possesses high biological activity.

It was thus shown for the first time that animal bone marrow contains a biologically active low-molecular-weight glutamylpeptide with abnormal optical activity compared with ordinary natural peptides. The isolated peptide is similar in its physicochemical properties (several electrophoretic and chromatographic mobilities, molecular weight, amino acid composition, optical activity) and biological activity to the peptide discovered previously in meristematic tissue of plants.

LITERATURE CITED

1. J. Bailey, Methods in Protein Chemistry [Russian translation], Moscow (1965), p. 37.
2. H. Determann, Gel Chromatography [Russian translation], Moscow (1970), p. 153.
3. A. M. Kuzin, A. I. Gaziev, V. A. Kopylov, et al., Radiobiologiya, 15, 124 (1973).
4. A. M. Kuzin, V. A. Kopylov, and A. I. Medvedev, Abstracts of Proceedings of Symposia of the Third All-Union Biochemical Congress [in Russian], Riga (1974), p. 323.
5. Yu. A. Mantsygin and A. M. Bagrova, Biol. Nauki, No. 12, 96 (1969).
6. A. I. Medvedev, V. G. Ladygina, and A. M. Kuzin, Dokl. Akad. Nauk SSSR, 203, 1408 (1972).
7. A. I. Medvedev, V. G. Ladygina, I. F. Medvedeva, et al., in: Mechanisms of Radiation Injury and Repair of DNP [in Russian], Pushchino (1972), p. 169.
8. M. L. Tiffany and S. Krimm, Biopolymers, 11, 2309 (1972).