CYTOTOXIC EFFECT OF METHIONINE-γ-LYASE ON CANCER CELLS IN CULTURE

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UDC 616.006-008.939.633.2-02:
615.355:577.152.351

KEY WORDS: methionine-γ-lyase; ovarian carcinoma; Fisher's lymphatic leukemia; antiproliferative action; DNA synthesis.

A search for new enzyme preparations with antitumor activity is going on at the present time [1]. It has been shown [3-5] that preparations of methionine-γ-lyase obtained from microorganisms have antiproliferative activity both *in vitro* and *in vivo*.

The object of this investigation was to study the effect of methionine-γ-lyase from *Pseudomonas putida*, obtained by a modified method, on DNA synthesis in cells of Fisher's lymphatic leukemia (cells of line L-8) and ovarian carcinoma (cells of line CaOv).

EXPERIMENTAL METHOD

CaOv cells were grown under standard conditions [2] in medium 199. For the experiment, CaOv cells were seeded in glass flasks 2 cm in diameter and grown for 24 h at 37°C. Each sample contained $200\text{-}300 \cdot 10^3$ cells in a volume of 2 ml. At the beginning of the experiment the enzyme preparation was added to the nutrient medium of the samples in a certain concentration in minimal volume (10-20 μl) and incubated for 24 h at 37°C. Labeled precursor of DNA synthesis, [^{3}H]thymidine (specific activity 12 Ci/mmole), was added in a volume of 20 μl to the medium containing the specimens 1 h before the end of incubation in a final concentration of 1 μCi/ml. Nucleic acid synthesis was stopped by placing the samples in ice. The medium was then decanted from the samples and the cells washed with Hanks' solution and 2.5% $HClO_4$, and then hydrolyzed in 5% $HClO_4$ for 20 min at 80°C.

Cells of line L-8 (a standard suspension culture of mouse leukemic cells was obtained from two DBA_2 males on the 12th day after intraperitoneal injection of cells of strain L-5178Y, by T. P. Ivanova et al.) were grown in medium RPMI-1640, containing 10% embryonic calf serum, 0.06% glutamine, and 0.008% gentamycin. Before the experiment the L-8 cells were seeded in glass flasks 2 cm in diameter at the rate of $200\text{-}300 \cdot 10^3$ cells in a volume of 2 ml. The enzyme preparation was added to the medium containing the samples 24 h after seeding, in minimal volume (10-20 μl), and incubated for 24 h at 37°C. The degree of DNA synthesis in the samples was determined under the conditions specified above. The samples were placed in ice 1 h after addition of [^{3}H]thymidine, centrifuged for 10 min at 1200 rpm, and the cell residue was washed consecutively with Hanks' solution and 2.5% $HClO_4$, under the same conditions of centrifugation, and hydrolyzed at 80°C for 20 min.

The digests of both types of cells were cooled, samples of 0.1 ml were taken from each digest, treated with ZhS-8 scintillation fluid, and counted in a liquid scintillation counter (Mark III, from Nuclear Chicago, USA). DNA synthesis in CaOv and L-8 cells was determined from the radioactivity in samples of the digests and measured in cpm/sample.

The experimental results were presented as arithmetic mean values calculated for 6-9 determinations. Differences are significant at $P \leqslant 0.05$.

EXPERIMENTAL RESULTS

After exposure of L-8 cells to different concentrations of methionine-γ-lyase inhibition of DNA synthesis was observed. Dependence of inhibition of DNA synthesis in L-8 cells on the

Department of Biochemistry, P. Lumumba Peoples' Friendship University. All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. Institute for Chemical Research, Kyoto University. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 5, pp. 87-88, May, 1983. Original article submitted July 13, 1982.

 0007-4888/83/9505-0672$07.50

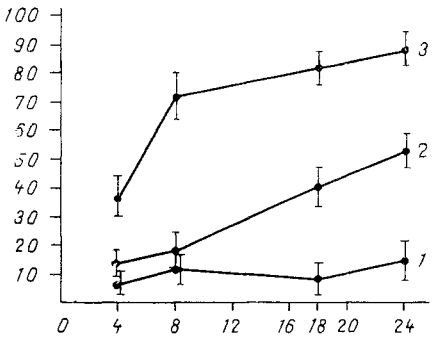

Fig. 1. Inhibition of DNA synthesis in L-8 cells depending on incubation time with various concentrations of methionine-γ-lyase. Abscissa, incubation time (in h); ordinate, inhibition of DNA synthesis (in %). Curves 1-3 correspond to enzyme concentrations of 0.1, 1.0, and 10 IU/ml.

TABLE 1. DNA Synthesis in CaOv Cells Depending on Incubation Time with Different Concentrations of Methionine-γ-lyase (M ± m)

Concentration of enzyme, IU/ml	Incubation time, h			
	4	8	18	24
	incorporation of [^{3}H]thymidine, cpm/sample			
Control	13 003±978	12 743±892	14 001±1120	10 068±706
10	13 807±694	11 888±874	13 371±966	13 301±710
1	14 620±972	11 022±660	15 180±1203	12 952±905

duration of incubation with different concentrations of methionine-γ-lyase is illustrated in Fig. 1. With a concentration of methionine-γ-lyase in the medium containing the samples of 10 IU/ml, inhibition of DNA synthesis developed rapidly and, after an exposure of 4-8 h, it reached 36-72%, respectively. Incorporation of [^{3}H]thymidine was reduced by only 16% between exposures of 8 and 24 h.

With the enzyme preparation in a concentration of 1 IU/ml inhibition of DNA synthesis developed more slowly than with a concentration of 10 IU/ml, and inhibition after exposures of 4-8 h amounted to 25-34%, respectively, of the inhibition observed after 24 h.

In a concentration of 0.1 IU/ml the enzyme had virtually no effect on incorporation of [^{3}H]thymidine into L-8 cells.

Dependence of incorporation of [^{3}H]thymidine into CaOv cells on the methionine-γ-lyase concentration is shown in Table 1. Clearly the enzyme had no effect on DNA synthesis in CaOv cells.

A preparation of methionine-γ-lyase obtained from *Pseudomonas putida* by a modified method thus has a marked action on DNA synthesis in a culture of mouse leukemia L-5178Y but has no such action on HeLa-like cells of the CaOv line.

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