CYTOSTATIC EFFECT OF L-LYSINE-α-OXIDASE FROM *Trichoderma harzianum* RIFAI AND *Trichoderma viride*

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KEY WORDS: ovarian carcinoma; DNA; RNA; protein synthesis.

Enzymes of micro-organisms are finding an ever widening application as medical preparations [1, 4, 7]. A special place is occupied by the use of enzymes of bacterial origin in oncology [2, 5]. One antitumor enzyme is L-lysine-α-oxidase (LO), the inhibitory effect of which on growth was first demonstrated in 1979 [6].

This paper gives comparative data on the effect of the new antitumor enzyme LO, obtained from a Soviet strain of *Trichoderma harzianum* Rifai and from *Trichoderma viride* (from Japan) on DNA and RNA synthesis in human ovarian carcinoma cells (line CaOv) in culture and also the results of the action of LO from *Tr. harzianum* Rifai on protein synthesis.

EXPERIMENTAL METHOD

A homogeneous preparation of LO from *Tr. harzianum* Rifai with specific activity of 29 U/mg was obtained by the method developed in the Department of Biochemistry of the Patrice Lumumba Peoples' Friendship University, and a homogeneous preparation of LO from *Tr. viride* I244-2 (Japan) with specific activity of 45 U/mg was obtained from the Institute of Chemical Research, Kyoto University. The test object for comparative study of the effect of the enzymes from these two sources on nucleic acid and protein synthesis consisted of cultures of HeLa-like cells of the CaOv line [3]. The cells were grown in a monolayer in medium 199 containing 10% bovine serum. For the experiment the cells were seeded in glass flasks (diameter 2 cm) and grown for 24 h at 37°C. Each sample contained $200 \cdot 10^3$–$300 \cdot 10^3$ cells in 2 ml of medium. The enzyme was then added to the incubation medium of the samples in a certain concentration and in minimal volume (100 µl) and the mixture was incubated for various times at 37°C. Specific precursors were added to the samples 1 h before the end of the incubation time: ^{3}H-thymidine (^{3}H-T, specific activity 1960 TBq/mole), the precursor for DNA synthesis, ^{3}H-uridine (^{3}H-U, specific activity 1026 TBq), for RNA synthesis, and ^{3}H-leucine (^{3}H-L, specific activity 244 GBq/mole) for protein synthesis, in a volume of 20 µl and in a final concentration of 37 MBq/ml. Nucleic acid and protein synthesis was stopped by putting the samples in ice. The cells were washed with Hanks' solution and 2.5% $HClO_4$ and hydrolyzed in 5% $HClO_4$ for 20 min at 80°C. Samples of the digest, measuring 0.1 ml, were added to ZHS-8 scintillator. The level of radioactivity in the samples was measured on a Nuclear Chicago Mark III liquid scintillation counter (USA) and expressed in cpm. The experimental results are given and the arithmetic mean of 6-9 determinations ± the standard deivation. Differences between values are significant at the $p \leq 0.05$ level.

EXPERIMENTAL RESULTS

During exposure of cells of the CaOv line to the enzyme inhibition of DNA, RNA, and protein synthesis was observed (Figs. 1, 2, and 3). Investigation of dependence of DNA synthesis in CaOv cells on the duration of incubation with LO from the two sources within the concentration range 10^{-2}–10^{-5} U/ml showed maximal inhibition of ^{3}H-T incorporation after 8 h (Fig. 1a, b). The enzyme concentration at which ^{3}H-T incorporation was about 50% of the control (EC_{50})

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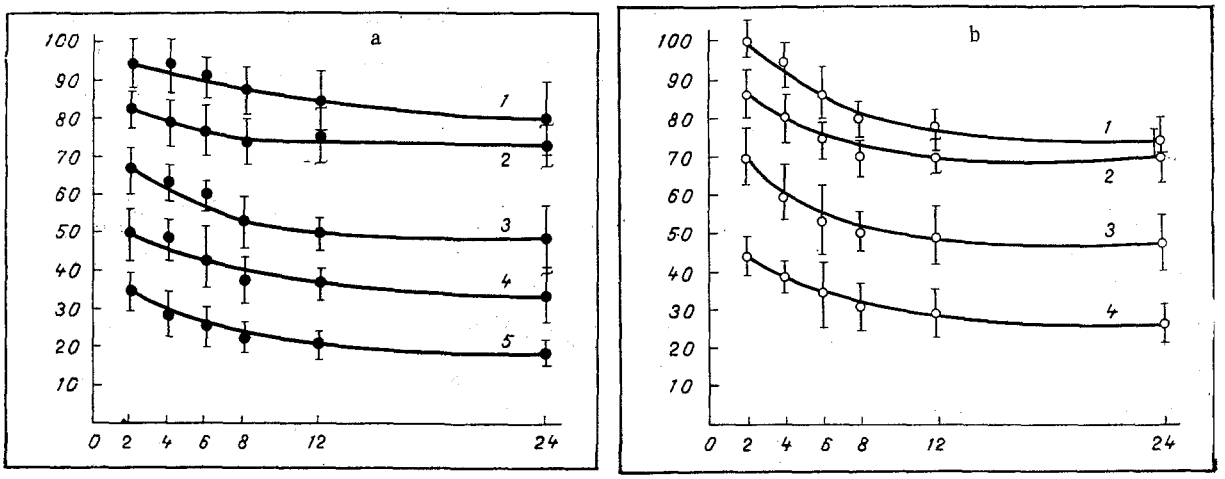

Fig. 1. Incorporation of ^{3}H-T in CaOv cells depending on duration of incubation with various concentrations of LO derived from *Tr. harzianum* Rifai (a) and *Tr. viride* (b). a: Enzyme concentration (in U/ml): 1) 10^{-1}, 2) 10^{-2}, 3) 10^{-3}, 4 10^{-4}, 5) 10^{-5}; b: 1) 10^{-2}, 2) 10^{-3}, 3) 10^{-4}, 4) 10^{-5}. Abscissa, incubation time (in h); ordinate, incorporation of ^{3}H-T (in % of control).

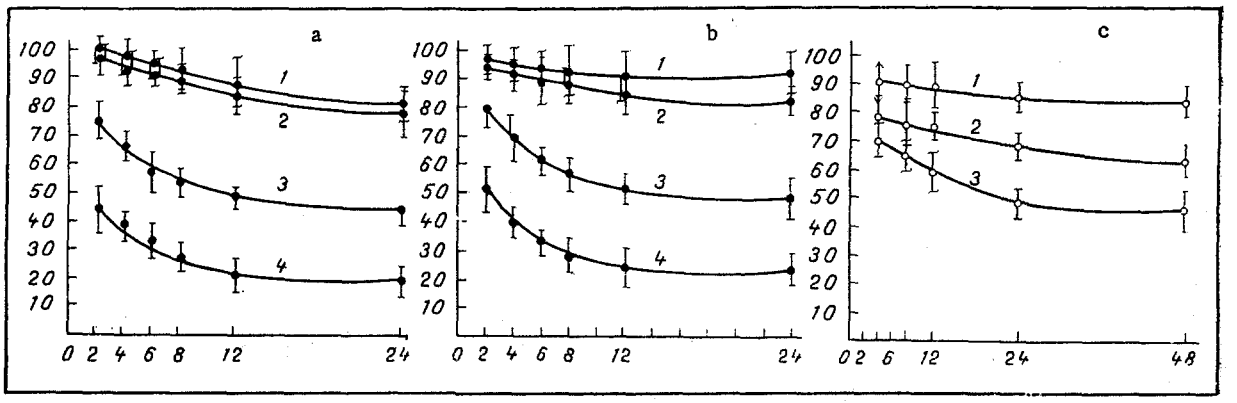

Fig. 2. Incorporation of ^{3}H-U (a, b) and ^{3}H-L (c) in CaOv cells depending on duration of incubation with various concentrations of LO from *Tr. harzianum* Rifai (a, c) and *Tr. viride* (b). Abscissa, incubation time (in h); ordinate, incorporation of label (in % of control). Enzyme concentration (in U/ml): 1) 10^{-2}, 2) 10^{-3}, 3) 10^{-4}, 4) 10^{-5}.

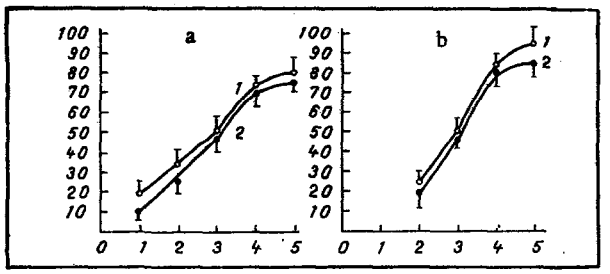

Fig. 3. Dependence of incorporation of ^{3}H-T (a) and ^{3}H-U (b) in CaOv cells on concentration of LO from *Tr. harzianum* Rifai (1) and *Tr. viride* (2). Abscissa, log of concentration (in U/ml); ordinate, incorporation of label (in % of control).

was 10^{-3} U/ml. In lower concentrations (10^{-4} and 10^{-5} U/ml) changes in the rate of DNA synthesis were not significant.

Inhibition of incorporation of ^{3}H-U in CaOv cells reached its highest values after incubation for 12 h with the enzyme from both sources (Fig. 2a, b). About 50% of inhibition was observed in a concentration of 10^{-3} U/ml, just as in the case of DNA synthesis.

Inhibition of ^{3}H-L incorporation in the presence of LO from *Tr. harzianum* Rifai was not significant, and the highest value of inhibition was obtained after incubation for 24 h (Fig. 2c).

The final values of inhibition of DNA and RNA synthesis in the presence of enzymes from the two sources was shown to be virtually a linear function of enzyme concentration (Fig. 3a, b).

Preparations of LO from the Soviet producer strain of *Trichoderma harzianum* Rifai and from the Japanese strain *Trichoderma viride* Y244-2* thus has a similar inhibiting action on the processes studied.

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CHANGES IN ANTITUMOR RESISTANCE OF HAMSTERS FOLLOWING EXPERIMENTAL REMOVAL OF UNFIXED MACROPHAGES

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Many investigations have demonstrated the important role of macrophages in the development of resistance to tumors [1-3, 5, 7]. The mechanisms by which macrophages kill human cells are not clear. However, direct contact between effector macrophages and target cells is probably necessary. The number of macrophages in the tissue of a growing tumor is known to correlate negatively with the formation of spontaneous metastases [6, 8]. It is accordingly natural to suppose that experimental removal of mobile macrophages (and other effector cells interacting with macrophages) from the location of tumor cells could significantly modify the antitumor resistance of the animal.

The aim of this investigation was to test this hypothesis.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred Syrian hamsters reared at the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR. Transplantable sarcoma Ad-12, induced initially in hamsters by human type 12 adenovirus, was used as the test tumor. The transplantation test was used in Murka's modification [4]. Hamsters of the experimental and control groups were inoculated subcutaneously with sarcoma Ad-12 cells at four points of their body in the following doses: 10^2, $2 \cdot 10^2$, 10^3, and 10^4. The experimental results were assessed by means of the following parameters: the latent period of appearance of tumors and the percentages of positive inoculations.

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