

COMPARISON OF THE EFFECT OF L-LYSINE- α -OXIDASE FROM *Trichoderma harzianum*
RIFAI AND *Trichoderma viride* ON NUCLEIC ACID SYNTHESIS IN HUMAN TUMOR CELLS
IN VITRO

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Enzymes of microorganisms are being increasingly used as medicinal preparations [1, 4, 7]. A special place is occupied by the use of enzymes of bacterial origin in oncology [2, 5]. One promising antitumor enzyme is L-lysine- α -oxidase (LO), whose growth-inhibiting effect was first demonstrated in 1979 [6].

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

EXPERIMENTAL METHODS

A homogeneous preparation of the enzyme LO from *T. harzianum* Rifai with specific activity of 29 U/mg was obtained by a method developed in the Department of Biochemistry, Patrice Lumumba Peoples' Friendship University, and another homogeneous preparation of LO from *T. viride* Y244-2 (Japan) with specific activity of 45 U/mg was obtained from the Institute of Chemical Research, Kyoto University. As the object used for comparative study of the effect of the enzymes from these two sources on nucleic acid and protein synthesis, cultures of HeLa-like cells of strain CaOv [3] were used. 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 $200 \cdot 10^3$ to $300 \cdot 10^3$ cells in 2 ml of medium. The enzyme was then added to the incubation medium of the samples in a definite concentration and in minimal volume (100 μ l) and incubated for various times at 37°C. Specific precursors were added 1 h before the end of the incubation time to the samples: ^3H -thymidine (^3H -T, specific activity 1960 TBq/mole) as precursor for DNA synthesis, ^3H -uridine (^3H -U, specific activity 1026 TBq) as RNA precursor, and ^3H -leucine (^3H -L, specific activity 244 GBq/mole) as protein precursor, in a volume of 20 μ l and in a final concentration of 37 MBq/ml. Nucleic acid and protein synthesis was stopped by placing 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 placed in ZhS-8 scintillation fluid. The level of radioactivity in the samples was measured on a Nuclear Chicago Mark III scintillation counter (USA) and expressed in cpm. The experimental results are given in the form of arithmetic mean of 6-9 determinations \pm the standard deviation. Differences between the results were considered significant at the $P \leq 0.05$ level.

RESULTS

During exposure of the CaOv cells to the enzyme preparations inhibition of DNA, RNA, and protein synthesis was observed. Investigation of dependence of DNA synthesis in CaOv

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cells on incubation time with LO from the two sources, in concentrations of 10^{-2} - 10^{-5} U/ml, showed that inhibition of incorporation of $^3\text{H-T}$ was maximal after 8 h. The enzyme concentration with which incorporation of $^3\text{H-T}$ was about 50% of the control (CE = 50) was 10^{-3} U/ml. In lower concentrations (10^{-4} and 10^{-5} U/ml) the enzyme caused only minor changes in the rate of DNA synthesis.

Inhibition of $^3\text{H-U}$ uptake in CaOv cells reached peak values after incubation for 12 h with enzyme from both sources (Fig. 2A, B).^{*} About 50% inhibition was observed with a concentration of 10^{-3} U/ml, just as in the case of DNA synthesis.

Inhibition of incorporation of $^3\text{H-L}$ in the presence of LO from *T. harzianum* Rifai was not significant, and it reached maximal values after incubation for 24 h (Fig. 2C).

The final values of inhibition of DNA and RNA synthesis in the presence of enzyme from both sources were shown to depend in a virtually linear manner on enzyme concentration (Fig. 3A, B).

Thus LO preparations from the Soviet producer strain *T. harzianum* Rifai and from the Japanese strain *T. viride* Y244-2 have a similar inhibitory action on the processes studied.

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Figures omitted in Russian original — Editor.